INFLUENCE OF TEMPERATURE AND PHOTOPERIOD UPON IONIC REGULATION IN THE RAINBOW TROUT *Salmo gairdneri*

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"Whoever wishes to investigate
medicine properly, should proceed
thus; in the first place consider
the seasons of the year, and what
effect each of them produces, for
they are not all alike, but differ
much from themselves in regard to
their changes"

Hippocrates

ABSTRACT

Interactions of photoperiod and temperature upon water-electrolyte balance were examined in rainbow trout acclimated to six combinations of two photoperiods (18h light: 6h dark, 6h light: 18h dark) and three temperatures (2, 10 and 18 °C). The influence of temperature and photoperiod upon plasma, skeletal muscle, cardiac muscle and liver levels of sodium, potassium, magnesium, calcium, chloride, water content, water distribution and cellular ion concentrations was determined by a one way analysis of variance. Significant ($p < 0.05$ or better) temperature effects at common photoperiods were observed in 70% of the analyses performed, showing no bias toward either photoperiod. Significant photoperiod effects occured in 57% of the analyses performed at common temperatures. The influence of photoperiod was most prevalent at reduced temperatures. Potassium and magnesium appeared to be particularly thermosensitive, while sodium and calcium were the most photosensitive of the electrolytes. The ionic composition of all tissues studied were relatively thermosensitive, with liver apparently being the most sensitive. On the other hand; the ionic composition of skeletal and cardiac muscle appear to be the most photosensitive of the tissues examined. Water content and distribution in skeletal muscle and liver were significantly influenced by temperature in 50% of the analyses performed showing a very strong bias toward "winter" animals. Photoperiod effects were significant in 56% of the water parameters measured with a strong bias toward the two lower temperatures.

Body weight was of significant influence in 16% of the 174

analyses performed. These data are discussed in terms of the effect of temperature upon ionregulatory mechanisms and the possible impact of photoperiod variations on endocrine systems influencing water-electrolyte metabolism.

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INTRODUCTION

Although it is one of the most intensively investigated areas of teleostean physiology, research into osmotic and ionic regulation often produces diverse and frequently controversial results. In part at least some of the discrepancies may stem from intrinsic variations in the stocks employed, and differences in the conditions under which the animals are maintained during experimentation. The present study attempts to evaluate the effects of two environmental conditions, temperature and photoperiod, as well as the intrinsic factor, specimen weight, upon the water-electrolyte status of rainbow trout, Salmo gairdneri.

In satisfying temperature-induced increases in oxygen demand the teleost may invoke adaptive responses at the hematological or the branchial exchanger system levels. Present evidence indicates that hematological responses are of supplementary importance at best under such circumstances (see Houston, 1973 for review). While little attention has been focused directly upon the activities of the branchial exchanger system during the thermoacclimatory process, several authors have considered the responses of fish to steadily increasing temperatures, or short-term exposure to circumstances prompting increased oxygen demand (Stevens and Randall, 1967 (a) (b); Davis, 1968; Randall, 1968; Randall et al., 1968). These studies suggest that satisfaction of elevated oxygen requirements in salmonid fishes is achieved primarily through compensatory increases in ventilatory flow, cardiac output; and effective branchial exchange area. Increases in water influx and electrolyte efflux are also associated with

these adjustments in the branchial exchanger system (Evans, 1969; Isaia, 1972; Motais and Isaia, 1972; Maetz, 1972; Stevens, 1972; Randall *et al.*, 1972; Wood and Randall, 1973 (a) (b)). Increased endoosmosis, for example, is associated with increases in glomerular filtration and urine flow rates, (Isaia, 1972; Motais and Isaia, 1972; Houston, 1973), and with increases in renal salt loss (Houston, 1973; Mackay, 1973). Renal salt loss, however, actually represents only 1/3 to 1/2 of the total electrolyte loss (Fromm, 1968) the remainder occuring by way of the gills.

Photoperiodic effects upon water-electrolyte status are to be anticipated as well. Roberts (1964) has demonstrated that photoperiod influences oxygen demand. Thus photoperiod *per se*, may affect water-electrolyte exchange rates in a fashion similar to temperature. From another viewpoint water-electrolyte balance is subject to regulation by a number of endocrine systems which are themselves influenced by photoperiod. Photoperiodic stimulation of the hypophysis - possibly as a consequence of pineal activation has, for example, been well demonstrated, as have the effects of pituitary hormones on electrolyte balance. (Lam and Hoar, 1967; Ball, 1969; Lam, 1969; Maetz, 1969; Olivereau and Ball, 1970; Pickford *et al.*, 1970 (a); McKeown and Peter, 1976). Other endocrine systems, notably the adrenal, corpuscle of Stannius,interrenal and the ultimobranchial glands have also been reported to be photosensitive and are directly or indirectly involved in water-electrolyte regulation (Ogawa, 1968; Chan, *et al.*, 1968; Chan *et al.*, 1969; Maetz, 1969; Pang, 1971; 1973; Bailey and Fenwick, 1975 (a) (b)).

There is some evidence suggesting that body weight also affects water-electrolyte status in teleost fishes. Oxygen

consumption per unit gill area tends to decline as body weight increases (Hughes, 1972; Nordie and Leffler, 1975). Increases in body size are also related to decreases in specific gill area ($mm^2.g^{-1}$) (Muir and Hughes, 1969; Hughes, 1972, Hughes et al., 1973), and Evans, (1968) found that tritiated water flux rates vary as a function of body weight. Present reports on weight-specific variations in electrolyte levels are, however, few and inconclusive.

Little, as yet, has been done in determining the extent to which photoperiod, temperature and specimen weight interact in governing water-electrolyte balance. In order to examine this aspect of the teleostean osmoregulatory process further, rainbow trout ranging in weight from 57 to 222 g have been investigated following acclimation to two photoperiods (18 hours light/6 hours darkness and 6 hours light/18 hours darkness) as well as three temperatures: 2°, 10° and $18^{\circ}C$. Consideration has been given to the influence of these factors plus the influence of specimen weight upon sodium, potassium, calcium, magnesium and chloride levels in plasma, skeletal muscle, liver tissue and cardiac muscle. Estimates of water content and distribution, and subsequent calculations of apparent cellular ion concentrations were made for liver and skeletal muscle.

Thus it was possible to:

(1) investigate the effect of temperature upon water-electrolyte balance under two distinct photoperiod regimes.

(2) investigate the effect of photoperiod upon water-electrolyte balance under three sets of temperature conditions.

(3) compare nominal "summer" (18°C, 18L/6D) and "winter" (2°, 6L/18D) specimens and

(4) evaluate the effect of photoperiod reversal in relation to nominal temperatures (i.e., 18°C, 18L/6D) vs. 18°C, 6L/18D and 2°C, 6L/18D vs. 2°C, 18L/6D).

REVIEW OF LITERATURE

INTRODUCTION

This review attempts to identify and evaluate literature contributing to the present views on temperature and photoperiod-related alterations in ionic and osmotic balance in teleosts. Initially consideration will be given to the adjustments a fresh-water fish undergoes as a result of changes in environmental temperature. Consideration will then be given to the role played by photoperiod, and to the possible involvement of hormonal systems.

The poikiothermic teleost confronted with increased environmental temperature, as would be the case under natural summer conditions, must satisfy increased oxygen demands (as suggested in the Belehradek equation[1] ($\text{Log } \dot{V}O_2 = K_o + K_i \cdot \text{Log } T$)) in the face of a decrease in dissolved oxygen at higher temperatures (as borne out by a reduced Bunsen Coefficient). Standard metabolism (measured by oxygen consumption ($\dot{V}O_2$) in goldfish and rainbow trout increase by 33- and 4-fold respectively when water temperatures are increased from lower to upper incipient lethal levels (i.e., from 0° - 42°C for goldfish, Carassius auratus, and from 0° - 24°C for rainbow trout, Salmo gairdneri), Fry and Hart (1957) and Florke et al., (1954 In Fry, 1957). In order to meet this increase in oxygen demand the teleost can (a) make adjustments in the branchial counter-current exchanger system (i.e., cardiac output, blood circulation, ventilation rate or volume, effective exchange area of the gill and/or diffusion pathlength), (b) enhance the oxygen carrying capacity of the

1. See Appendix page 114.

blood by increasing either the total number of erythrocytes or the amount of hemoglobin per erythrocyte, (c) shift the oxygen hemoglobin equilibrium curve by modulating hemoglobin-oxygen affinity. Each of these potential response forms will be considered:

(a) Adjustments in the Branchial Counter-Current Exchanger: Evans (1962), Hughes and Saunders (1970) and Davis and Cameron (1971) found that trout faced with a decreased supply of oxygen at higher temperatures increase ventilation rate prior to any increase in oxygen consumption. Similar results were also found in goldfish, Carassius auratus, (Rahn, 1966). However, this increased ventilation was inversely related ($p < 0.01$) with percentage oxygen utilization (Hughes and Saunders, 1970; Davis, 1971; Davis and Cameron, 1971). Hughes (Hughes and Saunders, 1970) suggested that the decrease in percentage utilization is largely due to an increase in the anatomical dead space in the gill which results from a higher percentage of the water failing to reach equilibrium with the blood. Since increases in ventilation volume and rate do enhance oxygen consumption but do so at the expense of extraction efficiency one is led to believe that there are other factors involved in satisfying increased oxygen demands at elevated temperatures.

Lingcod, Ophiodon elongatus, exposed to increasing temperatures respond by increasing cardiac output through increasing heart rate while maintaining a constant stroke volume (Stevens et al., 1972) whereas catfish, Ictalurus punctatus, and bass, Micropterus dolomieui, increased cardiac output by increasing stroke volume and maintaining a constant heart rate (Hart, 1957).

Perhaps the most consistently found responses to increased environmental temperature were decreased vascular resistance and increased blood circulation rates. Das and Prosser (1967) noted an increased blood supply to goldfish muscle at higher temperatures. Both sockeye salmon and rainbow trout displayed a reduced branchial vascular resistance when the fish were acclimated to higher temperatures (Davis, 1968, and Davis and Cameron, 1971). Potts and Morris (1968) found similar results in the antarctic ice fish Trematomus bernachii.

Since at higher temperatures rainbow trout increase oxygen consumption, gill perfusion, and cardiac output while at the same time showing a reduced arterial oxygen tension, Davis and Cameron (1971) suggested that this must reflect a significant degree of peripheral vasodilation, especially in the gill area. Without branchial vasodilation and subsequent reduction in oxygen tension, one would have anticipated an increased oxygen tension. Randall et al., (1972) found support for this conclusion in their observation that increased levels of circulating catecholamines occur in active Salmo gairdneri. Catecholamines known to cause vasodilation in teleosts caused a decrease in branchial vascular resistance and an increased blood flow in the gill. Randall (Randall et al., 1972) suggested that this increased blood flow through the gills could be a result of an increase in the effective exchange area of the gill due to an increase in blood flow through previously-unperfused secondary lamellae, the net result being increased gas exchange area and a reduced resistance to blood flow. Injections of adrenalin and

acetylcholine caused increased blood flow and vasodilation of the secondary lamellae and gill filaments respectively (Davis, 1971; 1972). This enhanced branchial blood flow reduces the mean diffusion pathlength of oxygen since the capillaries expand at the expense of the respiratory cell thickness, thus bringing the blood much closer to the oxygen providing water, as well as increasing the effective exchange area of the gill. These latter studies suggest obvious ways in which hormonal systems could play an active role in satisfying increased oxygen requirements. Their role will, however, be considered in more detail in the concluding portion of the literature review.

(b) Enhancement of the Blood-Oxygen Carrying Capacity: Studies have failed to prove that changes in the blood oxygen carrying capacity play a central role in the satisfaction of temperature-induced variations of oxygen demand. The influence of temperature does affect some species, but not all species in a similar fashion. Umminger (1971) found goldfish hematocrit to be lower at 18°C than at 10°C. In the same species Murachi (1959) found summer fish to have larger hematocrits than winter animals. He however, attributed the difference to a photoperiod rather than a temperature effect. Umminger and Mahoney (1972) found increased hemoglobin per cell in flounder held at higher temperatures . The rainbow trout shows some increase in hemoglobin, hematocrit and/or erythrocyte number following warm acclimation (DeWilde and Houston, 1967; Houston and Cyr, 1974; Murphy and Houston, 1977). Generally investigations with various species of fish have either indicated no significant change (Anthony, 1961; Linn, 1965; Falkner and Houston, 1966; Houston and DeWilde, 1968)

or significant increases in at least some hematological indices (Houston and Cyr, 1974) at different times of the year (Houston and DeWilde, 1968, Denton and Yousef, 1975).Denton and Yousef (1975) found adaptive responses to seasonal variations even at virtually constant water temperatures. The variable nature of the data leads one to believe hematological responses may rely upon a complex mechanism of which temperature is only one of the triggering agents.

(c) Adjustments in Oxygen-Hemoglobin Affinity:

The oxygen-hemoglobin equilibrium curve depicts the percentage saturation of hemoglobin with oxygen as a function of the partial pressure of oxygen (mm Hg). This is usually a sigmoid (s shaped) curve showing near saturation at oxygen tensions of 80 mm Hg and above. The steepest rise in the curve is between oxygen tensions of 10-60 mmHg, such that oxygen tensions above 60 mmHg yield a high percentage saturation. Decreasing the pH and/or increasing the carbon dioxide (CO_2) tension causes a rightward shift of the oxygen-hemoglobin curve (acid Bohr effect) and therefore at any given oxygen tension the hemoglobin will release a greater percentage of the oxygen. Thus when oxygenated blood encounters active tissues with high CO_2 levels and low pH values the hemoglobin will release the oxygen more readily to the tissue. The converse situation also exists (alkaline Bohr effect) in which the oxygen-hemoglobin equilibrium curve shifts to the left in an alkaline or high pH environment. This obviously would result in a greater retention of oxygen by the hemoglobin even at lower oxygen tensions.

Investigations upon the changes in the oxygen-hemoglobin equilibrium curve as a function of temperature are presently

inconclusive. Anthony (1961) and Black et al., (1966) found no adjustments made in response to temperature in goldfish, brook trout, Salvelinus fontinalis, and salmon. However, Grigg (1969) and Evans (1971) found a compensatory rightward shift of the oxygen-hemoglobin equilibrium curve at higher temperatures for both brown bullheads, Ictalurus nebulosus and rainbow trout, Salmo gairdneri. This shift would allow the blood to release more oxygen at higher temperatures. On the other hand, oxygen loading would be reduced only slightly by (a) this shift and (b) the reduction in environmental oxygen tension at higher temperatures since both the s-shaped character of the equilibrium curve and the relatively high level of oxygen tension in water even at higher temperatures would still allow for a high % saturation of the blood with oxygen.

In summary, teleosts confronted with an increased oxygen demand and/or a decrease in available oxygen appear to: (1) increase ventilation rate and volume (ii) increase cardiac output (iii) increase peripheral branchial vasodilation (possibly due to increased levels of circulating catecholamines) and (iv) decrease mean diffusion pathlength and increase effective gill area. This major adaptive response is probably supplemented by such positive compensatory adjustments as are made by some species in oxygen carrying capacity and oxygen-hemoglobin equilibrium curves.

If, however the trout satisfies its increased oxygen demand at higher temperatures in this fashion, secondary stresses are generated. Houston et al. (1968) defined this problem

"...Although efficacious in terms of oxygen uptake, such responses must prompt increases in endosmosis and salt efflux and lead to reductions in carbon dioxide tension. Accordingly, some modification in the activities or set points of the osmotic, ionic and acid-base regulating mechanisms might, a priori, be anticipated as correlates of the thermal acclimation process ....pp. 564" Thus at higher temperatures fishes responding in this way are inevitably subjected to increases in branchial ion efflux and water influx. Electrolyte loss via the gill is then compounded by loss via the kidney since freshwater teleosts typically respond to increased body water loading by increasing the glomerular filtration rate (G.F.R.) and urinary flow rates ($\dot{V}$). Q_{10} values for urine flow, for example, are between 2.20 and 2.40 for sucker, Catostomus commersoni, (MacKay and Beatty, 1968) and trout (Houston, 1973). Although urinary electrolyte concentrations tend to decrease at higher temperatures increases in urine flow lead to massive increases in urinary electrolyte losses under these conditions. Thus all electrolytes with the exception of potassium were excreted more rapidly at higher temperatures (Fromm, 1968). At higher temperatures the large increase in urinary flow rates of white sucker were attributed to an increase in the number of nephrons involved in urine production, while having no effect on tubular water reabsorption (Mackay and Beatty, 1968). Moreover, Hickman (1965) suggested that increases in the number of complete nephrons used cause reductions in water reabsorption. Fromm (1968) found electrolyte loss via the kidney approximated some 30% of the loss via the branchial epithelium of both trout and killifish.

Maintenance of Water Electrolyte Status in Freshwater Fish:

Freshwater teleosts must remain hyperosmotic to their environment. According to Maetz (1974) adaptation to a hypo-osmotic environment involves four basic requirements: (i) a decrease in water permeability of the body surface, (ii) a decrease in sodium and chloride permeability, (iii) the development of sodium and chloride uptake mechanisms and (iv) the development of a system to increase reabsorption of sodium and chloride in terminal portions of the kidney to allow for the excretion of excess water.

The main site of ion exchange in rainbow trout, Salmo gairdneri is the gill (Kerstetter et al., 1970). Studies by Krogh (1939), Fromm (1968) and Kirschner (1972 (a)) have established that the skin of rainbow trout does not participate to any measurable extent in ionic exchange. Consequently this review will be restricted to examination of ionic and osmotic regulation in the gill and kidney.

The gill, a multi-functional organ, is the site of gas exchange, nitrogenous waste clearance and mineral, water and acid-base balance. The gill possesses three distinguishable cell types: (1) 3-5 μ thick squamous respiratory cells which form much of the surface of the secondary lamellae and are responsible for gas exchange, (ii) round-to-oval shaped mucous cells (found in the inter-lamellar and filament regions) responsible for branchial mucous secretion, (iii) tall columnar, mitochondria- and tubular-rich "chloride" or Keys-Willmer cells found mostly on the afferent side of interlamellar regions and along the filaments. These cells are the site of electrolyte exchange in both freshwater and marine environments (Maetz, 1971).

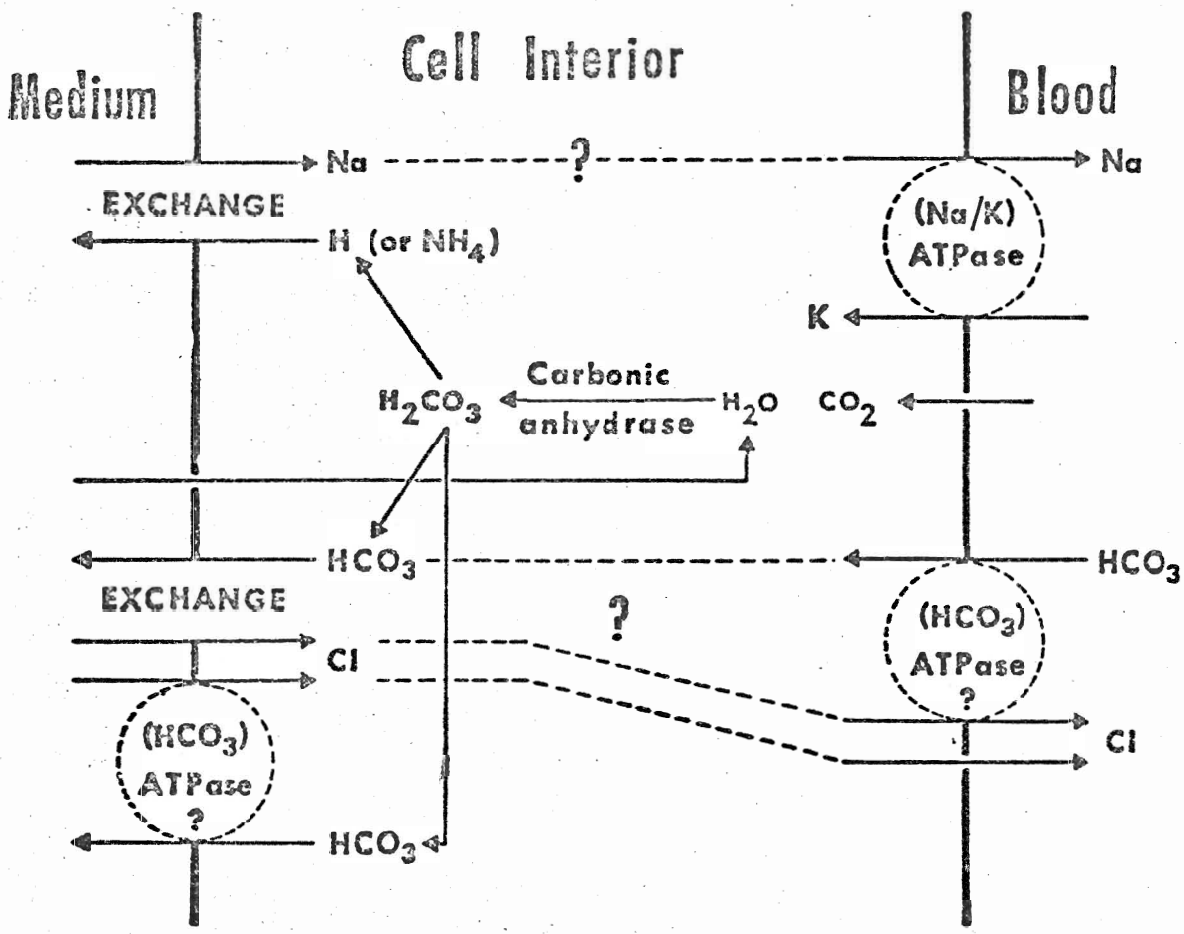
Branchial electrolyte regulation in freshwater teleosts involves two major processes: (i) exchange diffusion and (ii) active transport.

In discussing electrolyte regulation reference will be made to the schematic diagram of a Keys-Willmer cell (Maetz, 1971) on Page 23. Bearing in mind that at elevated temperatures the ventilation rate increases, one can easily see the immediate problem of electrolyte depletion from the blood across the respiratory lamellae to the environment and the subsequent problem of electrolyte recruitment. Kerstetter, Kirschner and Rafuse (1970) suggested that passive electrolyte loss occurs primarily across the thin and numerous respiratory cells.

(a) Exchange Diffusion

Smith (1953, cited in Maetz, 1971) determined that ammonia (NH_3) was the principal nitrogenous product of teleostian metabolism and was eliminated by the gills in freshwater fish. Subsequently, it was found that addition of ammonium (NH_4^+) to the environmental water inhibited ammonia clearance (as well as reducing Na^+ absorption) by the gills of goldfish (Maetz, 1971). Thus a potential Na^+/NH_4^+ exchange existed. Maetz (1971) gathered support for this Na^+/NH_4^+ exchange when he found that intraperitoneal injections of goldfish with NH_4SO_4 caused enhanced excretion of NH_4^+ as well as increased absorption of Na^+ by the gill tissues. In this study Maetz also suggested that the NH_4^+ excretion rate approximated the influx rates of Na^+. Similar support for a Na^+/NH_4^+ exchange was found for eels Anguilla anguilla (Garcia-Romera, Motais, 1966); flounder (Maetz, 1967) and rainbow trout (Kerstetter et al., 1970). deVooys (1968) dispelled the idea that Na^+/NH_4^+ exchange was

Figure 1. A schematic interpretation of the functional "chloride cell" as presented by Maetz (1971).


Medium
Cell Interior
Blood
Na
?
Na
EXCHANGE
H (or NH_4)
(Na/K) ATPase
K
Carbonic anhydrase
H_2CO_3
H_2O
CO_2
HCO_3
HCO_3
EXCHANGE
?
Cl
(HCO_3) ATPase ?
(HCO_3) ATPase ?
Cl
HCO_3

obligatory when carp, Cyprinus carpio placed in deionized water were found to continue to excrete NH_4^+ in the absence of any exchangeable Na^+. Subsequent transfer of these fish to tap water resulted in Na^+ uptake without augmentation of branchial NH_4^+ excretion. Maetz (1971) also found evidence to deny an obligatory Na^+/NH_4^+ exchange when acidification of the environmental water inhibited Na^+ uptake by the gill of goldfish. This was also substantiated in trout when the environmental water pH was reduced to 3.5. This resulted not in a reduction of Na^+ absorption, but rather an increased efflux ultimately resulting in death due to Na^+ depletion and damaging effects on gas exchange (Packer and Dunson, 1970). These results pointed to a possible Na^+/H^+ exchange. This possible exchange was substantiated further when Kerstetter et al., (1970) recognized that not only did the amount of Na^+ being absorbed exceed the amount of NH_4^+ being excreted, but that the pH of the environmental water also dropped considerably. In this work Kerstetter approximated that 1/3 of the Na^+ was being exchanged for H^+ ions. Maetz (1973 (a)) quantified this work by determining that Na^+ absorption in goldfish equalled the sum total of NH_4^+ and H^+ being excreted by the gill.

Two factors: (i) the presence of carbonic anhydrase in the gill Keys-Willmer cells (Leiner, 1938) and (ii) the fact that CO_2 is excreted via the gills, pointed to the possibility that bicarbonate (HCO_3^-) may be the exchange ion for chloride (Cl^-). Both Cl^- and Na^+ are depleted more rapidly to the environment at higher temperatures. The intracellular enzyme carbonic anhydrase

(C.A.) catalyzes the reaction of $CO_2 + H_2O \rightleftharpoons H_2CO_3$ (the water being absorbed from the environment, the CO_2 being given off by the blood). Carbonic acid (H_2CO_3) then proceeds by mass action to yield the products HCO_3^- and H^+ in a 1 to 1 ratio. The bicarbonate (HCO_3^-) ion was proven to be the exchange ion for Cl^- (see Maetz diagram) in two experiments with goldfish and trout. Addition of HCO_3^- to the external medium resulted in decreased Cl^- uptake by the gill, while intraperitoneal injection of HCO_3^- augmented Cl^- uptake in goldfish (Maetz, Garcia-Romeu, 1964) and in trout (Kerstetter and Kirschner, 1972; and Milne and Randall, 1976). Increases in external CO_2 or decreases in Cl^- caused increases in plasma HCO_3^- and plasma CO_2 respectively (Lloyd and White, 1967; Dejours, 1969).

Since the ultimate products of the carbonic anhydrase reaction, namely HCO_3^- and H^+ are probable exchange ions for Cl^- and Na^+, the possibility of an interdependence between Cl^- and Na^+ absorption was investigated. In the previously mentioned studies of Maetz (1971) in which Na^+ absorption in goldfish was either stimulated (injection of NH_4SO_4) or inhibited (addition of NH_4^+ to the environmental water) there were no noticeable effects upon Cl^- uptake. The addition of acetazolamide (an inhibitor of carbonic anhydrase) to the external medium reduced both Na^+ and Cl^- uptake (Maetz, et al., 1964). This was likely due to a reduction in the number of available exchange ions. Later work by Kerstetter et al., (1970); Kerstetter and Kirschner (1972) confirmed the inhibition of Na^+ uptake by acetazolamide but found no inhibition of Cl^- uptake in rainbow trout. Kerstetter speculated that HCO_3^- ions could diffuse down their concentration gradient from the blood into the cell and thereby provide exchange ions

for Cl^-, while the presence of acetazolamide still inhibited carbonic anhydrase and thus the release of H^+ ions. The net result would be a decrease in Na^+ uptake (Kerstetter et al., 1970), with no effect upon Cl^- uptake (Kerstetter and Kirschner, 1972). Recently, trout acclimated to higher temperatures have shown elevated levels of erythrocytic carbonic anhydrase. (McCarty and Houston, personal communication). This could provide increased concentrations of diffusable HCO_3^- ions in the blood. It appears that in freshwater fish branchial Na^+ and Cl^- uptake mechanisms may be related via carbonic anhydrase. This relationship is not obligatory, however, since the Na^+/NH_4^+ exchange system functions independent of carbonic anhydrase. In their 1972 study, Kerstetter and Kirschner postulated an active transport system for Cl^- uptake since movement was up an electrochemical gradient (inside the cell having a negative charge of -32mv).

(b) Active Transport:

The latter observation leads into the next area of discussion namely the involvement of active transport mechanisms in the ultimate recruitment of Na^+ and Cl^-. The two most prominent branchial active transport enzymes are: (1) sodium-potassium activated adenosine triphosphotase ((Na^+-K^+)-ATPase) and bicarbonate stimulated adenosine triphosphotase. ((HCO_3^-)-ATPase).

(i) (Na^+-K^+)-ATPase: Many authors have reported the existence of branchial (Na^+-K^+)-ATPase in salmon, Oncorhynchus tschawytscha, Oncorhynchus kisutch; Japanese and American eels, Anguilla japonica and Anguilla rostrata; rainbow trout, Salmo gairdneri; goldfish Carassius auratus; and killifish, Fundulus heteroclitus (Zaugg and McLain, 1972; Utida, Kamiya and Shirai, 1971; Butler and Carmichael, 1972; Sargent et al., 1975; Richards and Fromm, 1970;

Kerstetter et al., 1970; McCarty and Houston, 1977; Murphy and Houston, 1974; Epstein et al., 1968). Work by Utida et al., (1971) and Sargent et al., (1975) provides strong evidence for the existence of high levels of (Na^+-K^+)-ATPase localized within the "chloride" cells of eels. Sargent et al., (1975) also noted that the energy releasing enzyme succinic-dehydrogenase within the "chloride" cells increased at the same time as (Na^+-K^+) ATPase.

The active site of (Na^+-K^+)-ATPase was found to be the vascular border since addition of the (Na^+-K^+)-ATPase specific inhibitor ouabain to the environmental medium failed to inhibit the enzyme (Maetz, 1970; Richards and Fromm, 1970; Epstein et al., 1973; 1975). The probable mode of Na^+ recruitment in freshwater fish is by (i) exchange diffusion at the environmental border of Na^+ for either NH_4^+ and/or H^+ and once within the cell (ii) the Na^+ is then actively transported into the blood across the vascular border via the membranous (Na^+-K)-ATPase enzyme system. In this entire process (1) nitrogenous wastes and excess H^+ ions are excreted, (2) K^+ ions that have moved passively down their concentration gradient from the cell to the blood are returned to the cell and (3) Na^+ ions lost to the environment due to branchial efflux are restored to the plasma (Maetz, 1970; Kerstetter and Kirschner, 1972; Motais, Garcia-Romeu, 1972; Randall et al., 1972; Maetz, 1974).

(ii) (HCO_3^-)-ATPase:

(HCO_3^-)-ATPase was isolated from "chloride" cells by Sach (1970, cited in Maetz, 1971). Most studies indicate this enzyme to be active at the environmental border in freshwater fish "chloride" cells (Lloyd and White, 1967; Dejours, 1969; Epstein,

et al., (1973; 1975). Recently Kerstetter and Kirschner found this enzyme in trout to be both mitochondrial and microsomal, while admitting a likelihood of heavy cross-contamination of cellular fractions (Kerstetter and Kirschner, 1974). Maetz (1971) proposed that the (HCO_3)-ATPase at the environmental border functions quite similarly to the (Na^+-K^+)-ATPase at the vascular border by pumping HCO_3^- out and bringing Cl^- into the cell. The likelihood of this is suggested by the strong electrochemical gradient that exists at the cell/environment border (Kerstetter and Kirschner, 1972; 1974). Several authors found this membrane associated (HCO_3^-)-ATPase to be inhibited by SCN^- when added to the external media both in vivo (Epstein et al., 1974; 1975) as well as in vitro (Kerstetter and Kirschner, 1972; 1974). Epstein and co-workers found that SCN^- inhibited Cl^- transport and suggested SCN^- possibly competes for the Cl^- site of the carrier-mediated transport system. Rainbow trout acclimated to higher temperatures were found to maintain steady plasma Cl^- levels while microsomal (HCO_3^-)-ATPase activities increased over the range 2 to 18°C (McCarty and Houston, 1977). McCarty stated that this may reflect little more than non-adaptive thermal activation of this system. No relationship between Cl^- transport and branchial SCN^- sensitive (HCO_3^-)-ATPase was found by either Kerstetter and Kirschner (1974) or by Solomon et al., (1975). Kerstetter appears to favour the idea of Cl^- uptake being dependent upon large passive diffusion of HCO_3^- into the cell from the blood (Kerstetter and Kirschner, 1972). Whether or not this is the case has yet to be proven. However, once Cl^- ions are in the

cell they are believed to be taken into the blood by some, as for now unknown Cl^- pumping system located at the vascular border.

Renal regulation of electrolytes will be discussed the the section dealing with possible endocrine involvement.

Electrolyte Regulation as a Function of Temperature and Photoperiod:

A. Temperature:

Hickman et al., (1964) found that when rainbow trout acclimated at 16° were compared to those acclimated from 16° to 6°C that animals acclimated to the higher temperature had higher values of plasma Na^+ and K^+, while displaying lower plasma Cl^-, P_i and Ca^{++} levels. In this same study tissue Cl^- remained unaltered and Na^+ and K^+ varied inversely with temperature. Similar results were obtained by Rao (1969) for plasma Cl^- in resting trout and by Byrne et al., (1972) for plasma K^+ in Salmo salar. In contrast to the earlier work by Hickman et al., (1964), Houston et al., (1968) and Toews and Hickman (1969) both found little change in plasma Na^+ while Cl^- levels increased slightly at higher temperatures. They also noted decreases in plasma and tissue K^+. Houston et al., (1968) found plasma and tissue Cl^- levels in summer rainbow trout exceeded those of winter animals. McCarty recently found no significant increase in plasma Na^+, K^+ and Cl^- of rainbow trout acclimated from 2 to 18°C (McCarty and Houston, 1977).

Generally, within cyprinids, plasma Na^+ and Cl^- values were found to be either thermostable or elevated as in the cases of carp and goldfish (Houston et al., 1968; Prosser et al., 1970; Umminger, 1971; Umminger and Mahoney, 1972; Murphy and Houston, 1974). In the latter study, Murphy and Houston (1974) found that plasma Cl^- and Na^+

levels while remaining basically thermostable to display a peak level at an intermediate temperature. In that study Murphy found that branchial (Na^+-K^+)-ATPase activity increased linearly over the temperature range 5° to 35°C.

Most of the research indicates the salmonids and cyprinids appear to be able to maintain relatively stable electrolyte levels, over temperatures ranging from near upper to near lower incipient lethals in both groups.

Seasonal variations in electrolyte levels were observed by Houston et al. (1968, 1970). In winter carp plasma Na^+ levels exceeded those of summer and fall fish, while fall plasma Cl^- levels were higher than summer and winter values (Houston et al., 1970). Houston et al. (1970) also noted that the tissue water levels decreased with temperature with fall and winter fish and increased in summer fish. Smith and Ellory (1971), noted that intestinal (Na^+-K^+)-ATPase levels were greater in winter than summer goldfish. These results were likely due to incubation of the reaction mixtures at a common optimum temperature rather than at the environmental temperature. McCarty and Houston (1977) found incubation of this enzyme at the environmental temperature produced activities opposite those observed when incubated at a common optimum temperature for rainbow trout held at temperatures of 2°, 10° and 18°C. Although reports of seasonal influence on electrolyte balance are sparse they are related to both temperature and photoperiod.

B. Photoperiod:

To date no literature has dealt specifically with the influence of photoperiod upon water-electrolyte balance. However, research into endocrine involvement in electrolyte balance does

make reference to photoperiod modulation. These topics will be discussed in the final section of the literature review.

Water Content and Distribution

(a) Water Content: In goldfish the drinking rate increased 10 fold over the range 5° to 25°C (Isaia, 1972). Similar, but less pronounced changes also occur in freshwater eels (Motais and Isaia, 1972). These observations are against the generally accepted principle that a freshwater fish actually drinks very little but rather obtains water via the branchial epithelium (Maetz, 1971). Several authors have found no significant change in the water content of goldfish held at temperatures varying as much as from 5° to 25°C (Das, 1967; Heinicke and Houston, 1965; Prosser et al., 1970). Meyer et al. (1956) found direct transfer of goldfish to higher temperatures (thermal shock) caused decreases in both body and muscle water levels. Similar findings for temperature acclimation studies were made by Houston et al. (1970) for carp and Hickman et al. (1964) for rainbow trout. Houston et al. (1970) proposed a seasonal dependence since (1) in winter fish there was no significant difference in water content between fish held at 7° and 27°C, (2) summer fish displayed a direct relationship between temperature and water content and (3) fall fish displayed an inverse relationship between temperature and water content. Later, Houston (1973) stated that "...the muscle water content of animals held at common temperatures during different seasons of the year also varied significantly; the overall picture being one of a decline in muscle water content from summer through fall to winter....." The implication would be some sort of a seasonal influence upon temperature regulation of body water. In salmonid species

again several authors made somewhat consistent observations with brown and rainbow trout. Generally muscle and body water levels were lower at higher acclimation temperatures (Gordon, 1959; Hickman et al., 1964; Houston et al., 1968; Toews and Hickman, 1969). In Houston's study with trout (Houston et al., 1968) he noticed subtle differences between summer and winter fish, but both displayed an inverse relationship between temperature and water content. Fish (1963) substantiated a seasonal influence, when winter trout held at 8-9° had more or less consistently higher water content than summer trout held at 20-23°C. These results generally indicate that salmonids incur some degree of muscular hydration at lower acclimation temperatures.

(b) Water Distribution: The chloride space is the most commonly employed method of estimating the extracellular phase volume in fishes. Several authors found the chloride space to be thermostable in brown trout (Gordon, 1959), in rainbow trout (Hickman et al., 1964), in goldfish (Heinicke and Houston, 1965) and summer rainbow trout (Houston et al., 1968). The only cited cases in which the chloride space increased as a result of increased temperature were in rainbow trout brain tissue (Hickman et al., 1964), winter rainbow trout muscle (Houston et al., 1968) and trout muscle (Ogawa, 1975). Estimates of the cellular phase volume were either thermostable (Houston et al., 1968) or increased with temperature (Toews and Hickman, 1969). Contrary to this Hickman et al. (1964) found a seasonal increase in muscle chloride space of white sucker from fall to winter fish. In a later study Toews and Hickman (1969) found muscle chloride space in trout to increase both at temperatures above and below the intermediate temperature of 12°-14°C. Estimates of extra-

cellular phase volume by ^{14}C-sorbitol indicate a direct relationship with temperature, while cellular volumes decreased steadily with temperature (Prosser et al., 1970). This was determined to be a shift of water from the intracellular to the extracellular phase since tissue water remained constant.

Liver Tissue: Research into the influence of temperature upon water content in liver tissue is largely contradictary. Liver water content of goldfish was found to increase with temperature by several authors (Hoar and Cottle, 1952; Kanungo and Prosser, 1959; Das, 1967). Murphy (1961) found no temperature-related changes in water content of goldfish liver. Evans et al. (1964) implied the same was true for rainbow trout. Das and Prosser (1967) on the other hand found goldfish liver water content to decrease with temperature increases.

In summary, muscle water levels tended to be either thermostable, as was the general case in cyprinids, or inversely related with temperature as in the case of salmonids. While liver tissue seems to undergo some degree of hydration at higher temperatures the phase volumes, both cellular and extracellular, tend to have reciprocal relationships, with the latter tending to expand at the expense of the former during acclimation to higher temperatures. Strong evidence was also presented to indicate that water balance and regulation are at least season-sensitive.

Possible Hormonal Intervention in Iono-Osmoregulation

Considerable research has been devoted to determining the roles played by various hormones in ionoregulation by fishes. The following section summarizes work particularly pertinent to

the problem being considered in this study. Emphasis will be given to the effect of prolactin, cortisol, adrenocorticotrophic hormones, renin-angiotensin-aldosterone, growth hormones as well as the influence of the corpuscle of Stannius, pineal gland and the ultimobranchial gland upon electrolyte regulation and water balance in teleosts.

(a) Prolactin: Prolactin is secreted by the anterior pituitary upon being stimulated by the hypothalamus. One generally accepted role of prolactin in fishes lies in the reduction of electrolyte depletion. This is achieved by a reducing Na^+ efflux via the gills, in part through stimulation of lamellar mucous production (Ball and Ensor, 1965; Potts and Evans, 1966; Lam, 1968; Ball, 1969; Ball and Ensor, 1969; Lam, 1969; Leatherland and Lam, 1969; Maetz, 1969; Pickford et al., 1970 (a); MacFarlane, 1974). In the work of Lam and co-workers the sticklebacks used were physiologically hypophysectomized (hypohysis is virtually non-functional under summer conditions in a marine environment) while other fishes were surgically hypophysectomized (hypophysis removed). Prolactin injection of hypophysectomized fish has been shown to increase both the size, number and activity of mucous cells in the gills of goldfish and sticklebacks (Ogawa, 1970; Lam, 1969). This increased mucous secretion would decrease the permeability of Na^+ and thus reduce Na^+ loss that follows transfer of fish from salt to freshwater. Pickford et al. (1970 (a), (b)) found that prolactin injection of hypophysectomized killifish before transfer from salt to freshwater decreased the gill (Na^+-K^+)-ATPase activity while increasing that of the kidney. This response is likely to decrease extrarenal excretion of Na^+ as well as increase renal reabsorption of Na^+

as is necessary in adaptation to freshwater.

The mechanisms triggering production and/or secretion of prolactin appear to be related to changes in (a) photoperiod, (b) osmolarity of fluid bathing the prolactin secreting eta cells and (c) temperature. Increased photoperiod was found to increase secretion of prolactin (Lam and Hoar, 1967; Ball, 1969; Ogawa, 1970, 1975; McKeown and Peter, 1976). Decreases in osmolarity (primarily the concentration of Na^+ in freshwater fishes) of the fluid bathing the eta cells stimulates the production and secretion of prolactin (Maetz, 1967 (a); Lam and Leatherland, 1969; Ball, 1969; Ball and Ensor, 1969; Olivereau and Ball, 1970; Doyle and Epstein, 1972; Peter and McKeown, 1975). Recently McKeown and Hazlett (1975) found that transfer of salmon from salt to freshwater caused a preferential increase in labelled leucine incorporation into prolactin. Utida et al. (1971) also found freshwater eels to have 2.5 times the prolactin levels of their marine counterparts. Under natural conditions, increased photoperiods are normally accompanied by increases in environmental temperatures. McKeown and Peter (1976) noted that serum prolactin levels increased at the expense of pituitary prolactin levels, when goldfish were exposed to longer photoperiods; thus increasing circulation of the hormone. In the same study, these authors also found the circulating levels of prolactin to be substantially higher in goldfish at 20°C than those held at 10°C. Assuming, as described earlier, that fish exposed to increased temperatures do increase branchial ventilation, cardiac output and effective gill area, one can readily see the advantages of simultaneous increases in prolactin secretion as a compensatory response likely to reduce further Na^+ efflux that would be anticipated under these conditions.

Lam and Hoar (1967) and Lam (1969) recognized that prolactin prevented excess water loading in freshwater fish. Following transfer of the stickleback from seawater to freshwater, as with increases in water temperature, fish undoubtedly must take on extra water and consequently the problem of removing it. Regarding kidney function, Hickman (1965) found that there was an intermittancy in the number of functional glomeruli, such that not all glomeruli in teleosts are functional at the same time. He also noted that some glomeruli were operating with part of the glomerular capillaries constricted. This resulted in decreased filtration pressure and a subsequent decrease in urinary fluid production and flow. Decreased flow rates then increase time for water reabsorption due to increased physical contact with the tubular epithelium. Hickman continued his speculation to say that the greater the percentage of partial to fully functional glomeruli within the kidney the greater the water reabsorption. Lam and Leatherland (1969) observed that prolactin injections caused increases in the glomerular tuft diameter and a reduction in the ratio of partially to fully functional glomeruli. As postulated by Hickman (1965), Lam and Leatherland observed increased urine flow presumably due to increased filtration pressure which would increase the flow rate and decrease contact time with the distal tubular epithelium. Similar results to those observed by Lam and Leatherland (1969) were made by Stanley and Fleming (1967 a); Lam (1969); Potts and Fleming (1970); Olivereau and Ball (1970) and MacFarlane (1974).

It will be apparent from its effects upon the Na^+ permeability of the gill tissue and the degree of activity of glomeruli in the kidney and its sensitivity to temperature, photoperiod as well

as circulating levels of Na^+ that the pituitary and prolactin are intimately involved in Na^+ regulation and water balance in freshwater fish.

(b) Cortisol: Cortisol is produced in the adrenal gland and its production is stimulated by adrenocorticotrophic hormones secreted by the pituitary, which it (the pituitary) secretes when it is stimulated by the hypothalamus. Adrenalectomy (removal of the adrenal gland) of freshwater teleosts leads to decreases in net sodium influx. This is then ameliorated by subsequent injections of cortisol (Mayer and Maetz, 1967; Maetz, 1969; Chan et al., 1968 (a); Chan et al., 1969). In saltwater species this operation results in death or a very slow increase in Na^+ efflux, which again can be corrected by cortisol injections (Mayer and Maetz, 1967; Maetz, 1969). Saltwater adaptation induces an increase in interrenal activity, ACTH (adreno-cortiotrophic hormone) cell activity and subsequent cortisol production (Maetz, 1969; Olivereau and Ball, 1970). As expected hypophysectomy resulted in decreased plasma cortisol levels in rainbow trout (Donaldson and McBride, 1967). In similarly treated animals subsequent cortisol injections caused levels of branchial (Na^+-K^+)-ATPase activity to increase above those of control fish also in saltwater, (Butler and Carmichael, 1972; Kamiya, 1972) as well as an increase in Na^+ efflux and water absorption (Ball, 1969; Pickford et al., 1970 (b); Ogawa, 1975; Porthe-Nibelle and Lahlou, 1975). Findings similar to the latter were also obtained following ACTH injections (Olivereau and Ball, 1970; Ball, 1969). Intact freshwater eels injected with cortisol on a long term basis displayed increased euryhalinity (ability to live in waters having a wide range of salinities) associated with increased

branchial (Na^+-K^+)-ATPase activity equal to that of saltwater adapted eels (Epstein et al., 1971). In later work, increases in the number of "chloride" cells were observed in freshwater fish following similar treatment. However, these "chloride" cells failed to come into contact with the environmental border (therefore function in Na^+ efflux) until the fish were actually exposed to a hypertonic marine environment (Doyle and Epstein, 1972). Further proof that cortisol injection enhances euryhalinity was presented when freshwater Japanese eels, Anguilla japonica, injected with cortisol took on the silver colour characteristic of saltwater smolts (Kamiya, 1972). Salt-loaded freshwater rainbow trout increased Na^+ efflux and excretion when injected with cortisol or deoxycorticosterone. (Chester-Jones et. al., 1969).

It appears that both ACTH and cortisol are particularly important when the animal is in a hyperosmotic environment, or subjected to experimentally-induced hypernatremia. In both cases the net result is an increased Na^+ efflux. In freshwater fish prolactin clearly reduced net Na^+ efflux.

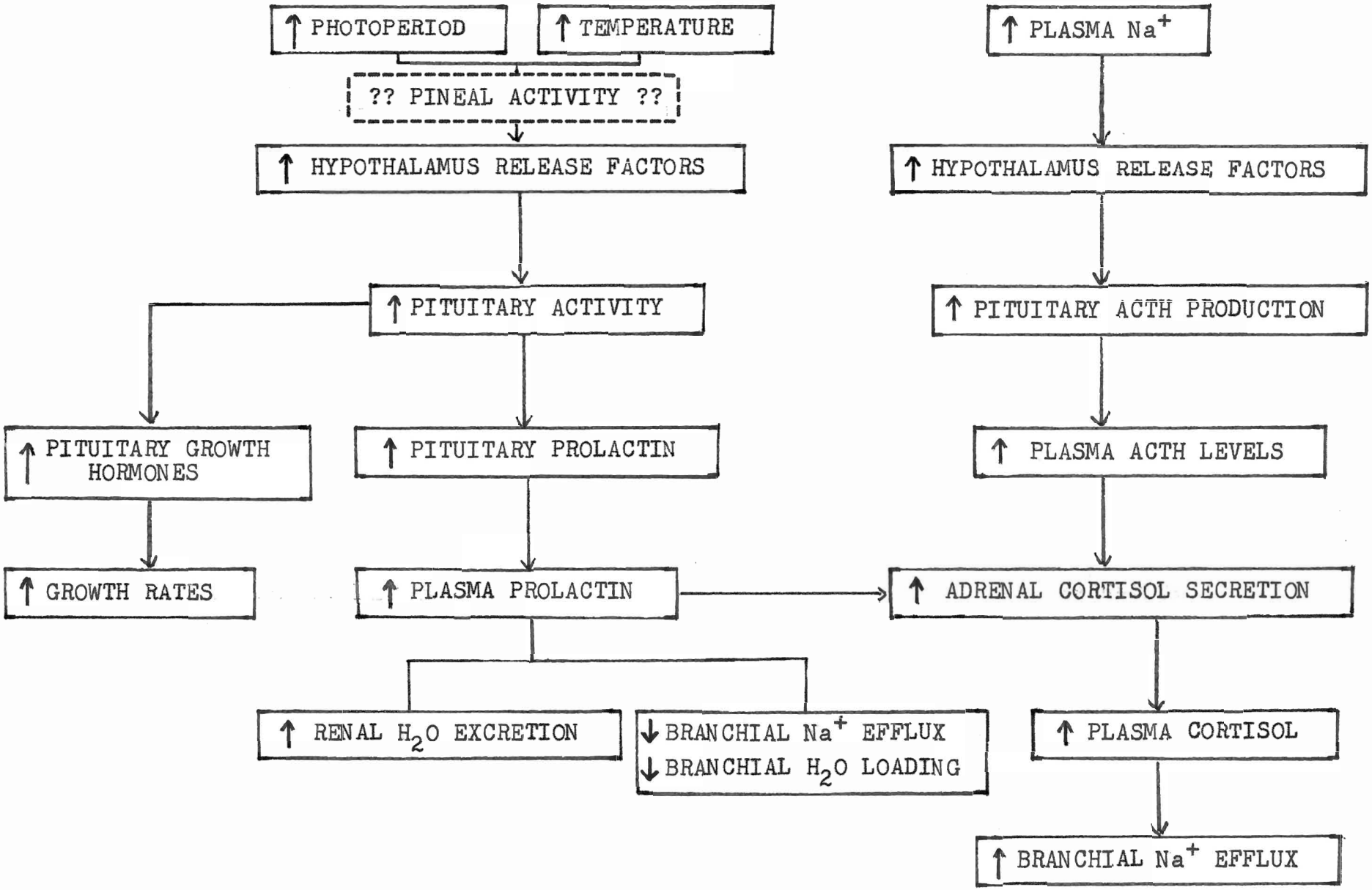
Potts and Fleming (1970) observed an increase in plasma cortisol levels when hypopysectomized killifish were injected with prolactin. Cortisol, when injected in small doses was also found to increase Na^+ absorption in freshwater fish. These findings may indicate a complex interrelationship between two distinct systems in freshwater fish.

Evidence for circannal, as well a temperature dependent variation in cortisol levels was found. Fryer (1975) found corticosteroid concentrations in goldfish to be higher in June even on a short photoperiod, than in November on a long photo-

period. Mahon et al. (1962) noted increased interrenal activity in goldfish exposed to higher temperatures. Ball (1969) and Ogawa (1975) found prolactin levels in sticklebacks and goldfish also to be higher in summer as opposed to winter animals. Thus both cortisol and prolactin appear to contribute to Na^+ regulation in freshwater fish and both appear to be sensitive to temperature, photoperiod and seasonal changes (Fig. 2.).

(c) Corpuscle of Stannius: The corpuscles of Stannius are an endocrine gland located on or in the mesonephric portion of the kidney of ray-finned fishes. This gland in freshwater fish appears to contribute to maintaining a hypocalcemic plasma since its removal results in immediate hypercalcemia (Ogawa, 1969; Chan et al., 1969; Leloup-Hately, 1970; Pang, 1971; Butler, 1972; Bailey and Fenwick, 1975 (a)). Stanniectomy (removal of the corpuscle of Stannius), being associated with a hypercalcemic response, also results in an overall decrease in both heart rate and in blood pressure (Chan et al., 1969; Bailey and Fenwick, 1975 (a)). The decrease in blood pressure as suggested by Bailey and Fenwick (1975 (a)) was likely brought about by a general decrease in cardiac electrical activity due to hypercalcemia. Since receptor sites on excitable membranes have a greater affinity for Ca^{++} than Na^+, a hypercalcemic environment could cause an inhibition of action potential propogation and thus decrease the rate of contraction of the heart. Secondly, high Ca^{++} concentrations tend to inhibit the uptake and binding of norepinephrine by cardiac muscle. The net result is a slowing of the heart rate and a subsequent decrease in blood pressure. Decreased blood pressure would also result from a loss of tonus of the blood vessels (thus decreased resistence to flow) and a general hyperpolarization of membranes of smooth muscle cells

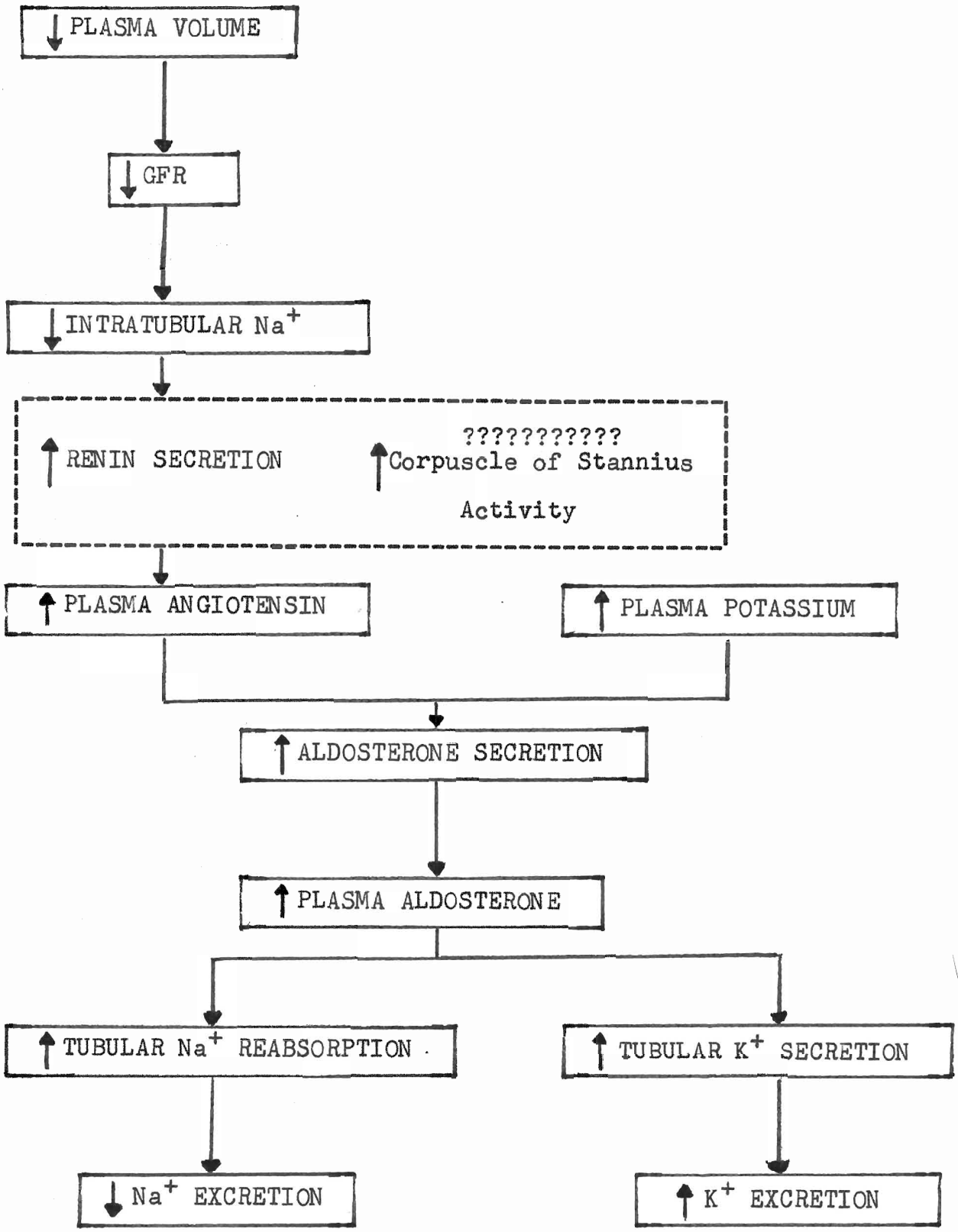
Figure 2. Pathway indicating possible hypothalamal involvement in osmo-ionoregulation in freshwater teleosts.


↑ PHOTOPERIOD
↑ TEMPERATURE
?? PINEAL ACTIVITY ??
↑ HYPOTHALAMUS RELEASE FACTORS
↑ PITUITARY ACTIVITY
↑ PITUITARY GROWTH HORMONES
↑ GROWTH RATES
↑ PITUITARY PROLACTIN
↑ PLASMA PROLACTIN
↑ RENAL H_2O EXCRETION
↓ BRANCHIAL Na^+ EFFLUX
↓ BRANCHIAL H_2O LOADING
↑ PLASMA Na^+
↑ HYPOTHALAMUS RELEASE FACTORS
↑ PITUITARY ACTH PRODUCTION
↑ PLASMA ACTH LEVELS
↑ ADRENAL CORTISOL SECRETION
↑ PLASMA CORTISOL
↑ BRANCHIAL Na^+ EFFLUX

(Bailey and Fenwick, 1975 (a)). The significance of this decrease in cardiac output and blood pressure lies in the reduction in filtration pressure and urine volume, which in turn will affect urinary electrolyte flux and water flow in the kidney (Chan et al., 1969). Injections of angiotensin II or the implanatation of corpuscles of Stannius (or its extract) into stanniectomized fish restores Ca^{++} balance, increases cardiac activity and blood pressure and alleviates the related effects on the kidney and its function. (Ogawa, 1968; Chan et al., 1969; Bailey and Fenwick, 1975 (a)).

Angiotensin is produced by the action of renin on the plasma bound protein angiotensinogen. Renin production (the rate limiting step) is stimulated by low intratubular Na^{+} levels in the kidney, which as previously mentioned would be affected by blood pressure and glomerular filtration. Once in the blood angiotensin stimulates the secretion of aldosterone by the adrenal cortex. Aldosterone then causes (a) increased tubular reabsorption of Na^{+} and (b) an increased tubular secretion of K^{+}. (Fig. 3). Bailey and Fenwick (1975 (b)), proceeded to determine whether the action of angiotensin and the secretion of the corpuscle of Stannius functioned similarly. They injected each separately into intact eels. Angiotensin II caused a dramatic increase in blood pressure for 10-15 minutes and a significant increase in plasma ionic calcium, while total plasma calcium decreased slightly. Injection of corpuscle of Stannius extract caused no effect on blood pressure, while decreasing plasma ionic Ca^{++} levels. This decrease was supposedly due to increased plasma protein binding of Ca^{++}. These results indicate that angiotensin II and the corpuscle of Stannius act differently. However,

Figure 3. Pathway indicating aldosterone regulation of sodium and potassium and a possible involvement of the corpuscle of Stannius.


↓ PLASMA VOLUME
↓ GFR
↓ INTRATUBULAR Na^+
↑ RENIN SECRETION
??????????
↑ Corpuscle of Stannius Activity
↑ PLASMA ANGIOTENSIN
↑ PLASMA POTASSIUM
↑ ALDOSTERONE SECRETION
↑ PLASMA ALDOSTERONE
↑ TUBULAR Na^+ REABSORPTION
↑ TUBULAR K^+ SECRETION
↓ Na^+ EXCRETION
↑ K^+ EXCRETION

Ogawa (1968) found that injection of angiotensin II had a hypocalcemic effect in stanniectomized goldfish, and was without effect on intact goldfish. This may indicate a species specific response to angiotensin II.

Davis (1962) postulated a functional renin-angiotensin-aldosterone system in teleost fishes. This idea gained further support when Porthe-Nibelle and Lahlou (1975) located detectable levels of aldosterone in goldfish plasma. Sokabe et al. (1970) made a link between the angiotensin system and the corpuscle of Stannius when his research located concentrations of renin in the corpuscles of Stannius as well as the kidneys of two cyprinids. The possibility of a corpuscle of Stannius-related renin-angiotensin-aldosterone system in fish is advanced further if one considers the results of Chan et al. (1969) who found plasma Ca^{++} levels increased, plasma Na^{+} levels decreased and plasma K^{+} increased as a result of stanniectomy. The effect on the latter two ions would be consistent with removal or decrease of circulating aldosterone levels in the kidney, if the corpuscle of Stannius regulates the renin-angiotensin-aldosterone system. As mentioned earlier the effects of stanniectomy were ameliorated by angiotensin II injection or implantation of the corpuscle of Stannius. It therefore appears that the corpuscle of Stannius may be related to a functional renin-angiotensin-aldosterone system. Both of these are somehow involved in Ca^{++} regulation and their role in Na^{+} and K^{+} regulation at the renal level cannot be ruled out.

(d) Ultimobranchial Gland: This gland is the counterpart of the mammalian thyriod "C" cells. Pang (1971) noted hypertrophy and hyperplosia of the ultimobranchial gland as a result of stanniectomy. Rasquin and Rosenbloom (1954) found similar results when exposing several teleosts to total darkness. They also noted extensive depletion of skeletal Ca^{++}. Copp et al. (1968) in studying calcitonin in non-mammals proposed that the ultimobranchial gland displays a hypocalcemic calcitonin-like activity in teleosts. Thus, the ultimobranchial gland may also be related to the corpuscle of Stannius in its Ca^{++} regulating role.

(e) Pineal Gland: The pineal is an extremely photosensitive gland located in the diencephalic region of the brain, just posterior to the thalamus. Pang (1973) found evidence that the pineal may be involved in Ca^{++} regulation. In his study 13% of all pinealectomized (removal of the pineal gland) killifish displayed non-significant decreases in mean plasma calcium levels. Sage and de Vlaming (1975) and Bailey and Fenwick (1975) found this organ to mediate hypothamic stimulation of the hormone secreting pituitary. They found that the photosensitive pineal may stimulate eta cell secretion of prolactin in response to alterations in photoperiod. If the pineal does influence the hypothalamus, then perhaps the other hormonal systems are at least affected, if not controlled by pineal photosensitivity.

(f) Growth Hormones: Growth hormones, also falling under pituitary control, were shown to be sensitive to photoperiod. Winter Salmo salar under long summer photoperiods displayed (i) an increased growth rate, (ii) silver smolt markings and (iii) a decreased coefficient of condition; all of which are associated with spring smolting animals (Komourdjian, Saunders

and Renwick, 1976 (a)). These authors also observed considerable hyperplasia and hypertrophy of the pituitary. They also found that injection of short day "winter" animals with porcine growth hormone induced responses similar to those observed in winter animals on a long photoperiod.

Thus, many hormones and hormone systems appear to be both individually and collectively involved to some extent in the regulation of water-electrolyte balance in teleosts. Although no experiments dealt directly with the effect of a specific hormone *per se* on electrolyte balance in thermally and photoperiodically acclimated fish, the implications of their involvement are undeniably present. Speculation as to how these hormones may have participated in iono-osmoregulation in this study in which rainbow trout, *Salmo gairdneri* were acclimated to 2°, 10° and 18°C under two photoperiods 18L/6D and 6L/18D will be considered in the discussion section of this paper.

MATERIALS AND METHODS

Species: Rainbow trout (Salmo gairdneri), ranging in weight from 57 to 227 g (mean = 101.3 g) were purchased in two lots (Dec. 27, 1973 and Mar.4, 1974) from a local commercial hatchery (Goosens Trout Farm, Otterville, Ontario).

Holding Conditions: Each lot was evenly separated and maintained in three fiberglass tanks (Frigid Units MT-700), each having a capacity of 600 litres. These tanks were equipped with false bottoms, constant volume overflow systems, and a double filtration system of urethane and activated charcoal. Recirculating pump-refrigeration units were operated against 500-W stainless steel immersion heaters coupled to Y.S.I. (Yellow Springs Instruments) thermoregulators to control water temperatures to within $\pm 1.0^{\circ}C$ of the desired value. An additional constant-operation recycling refrigeration unit(Neslab HX-50)was used to supplement cooling capacity in the $2^{\circ}C$-acclimation tank.

Sufficient dechlorinated St Catharines city water (Cal-Brook Water Filter) was added by way of an overflow system to provide for 2 to 3 full tank volume replacements daily. Urethane filters were cleaned daily, and fecal and undigested debris removed using a dip net. This, as well as periodic cleaning the tanks tended, through habituation to reduce adverse effects associated with netting fish at the time of sampling. (Umminger and Gist, 1973).

Water Quality Parameters: Temperature, dissolved oxygen, total hardness and alkalinity and pH were measured daily during acclimation. Mean dissolved oxygen levels as measured by a dissolved oxygen meter (Y.S.I. model 51A) were 13.5, 7.3 and

5.4 Mg $O_2 \cdot l^{-1}$ for 2°, 10° and 18°C respectively. Total hardness and total alkalinity determined by titration ("Standard Methods for the Analysis of Water and Wastewater", American Public Health Assoc.) were stable at mean values of 137.4 and 94.7, 136.6 and 95.0 and 135.2 and 91.8 Mg $CaCO_3 \cdot l^{-1}$ for the 2°, 10° and 18°C acclimation tanks respectively. Water pH was also stable at all temperatures, and averaged 7.4 (Fisher pH Meter).

Photoperiod: Each tank was provided with a top canopy and two 40 watt light receptacles producing 11-18 foot candles light intensity at the water surface. Light periods were controlled within ± 10 minutes by means of Intermatic T-101 time switches. Fish received in December were immediately placed on a 6 hour light - 18 hour dark regime (6L/18D) and hence represented "winter" day fish. Trout obtained in March were acclimated to "summer" day conditions (18 hours light - 6 hours dark; 18L/6D). All maintenance and sampling of both water and fish was carried out during light periods so as not to disrupt the photoperiod regime.

Temperature Acclimation: Specimens were held at origin temperature (~10°C) for one week to allow for recovery from transport stress and adjustment to the new environment. With the commencement of normal swimming and vigorous feeding water temperatures were adjusted by approximately 1°C/day increments to final acclimation levels (2°, 10° and 18° C). These conditions were then maintained for a minimum of two weeks prior to sampling.

Feeding: The fish were fed Purina Trout Chow (Ralston Purina

Co.) once daily at noon ad libitum, over a 30 minute period. Excess food and feces were then removed from the tanks.

Sampling: Food was denied on the day of sampling. The sampling procedure for each fish was as follows. The fish, having been habituated to the presence of nets, were captured and stunned quickly with a blow to the head region. Weights and lengths were recorded. Blood was immediately drawn into ammonium heparinized syringes via caudal puncture. Estimates of hematocrit were made using an Adams Microhematocrit Centrifuge prior to centrifugation of the remaining whole blood (5000 rpm, 5 minutes). Plasma was decanted off, and immediately frozen ($-12^{o}C$) in sealed plastic containers. Samples of epaxial skeletal muscle (minus skin, scale and bone) were taken anterior to the dorsal fin and divided into two pieces of approximately equal weight (1g). The ventricular portion of the heart was dissected free, flushed and blotted dry. The liver was removed and divided into three roughly equal portions. All tissue samples were placed in separate labelled and tared test tubes, sealed with parafilm and frozen ($-12^{o}C$) until tube plus tissue could be weighed to estimate wet tissue weight. Tubes were then resealed, and frozen until analyzed. Sex was determined by examination of gonadal tissue. The entire sampling procedure from netting to refrigeration of tissue and freezing of plasma required 12 to 15 minutes per fish.

Tissue Analysis:

(A). Chloride Determinations:[1] Chloride levels in skeletal muscle, liver tissue ($mM.kg^{-1}$) and plasma ($mEq.l^{-1}$) were estimated by automatic potentiometric titration with AgCl using a Cotlove Chloridometer. Tissue samples were digested in hot

1. See Appendix page 115.

sodium hydroxide and extracted with alkaline perborate.

(B). Sodium, Potassium, Magnesium and Calcium Determinations: [1]

These values were determined by atomic absorption spectrophotometry (Perkin Elmer 403) using the dilution technique of Paschen (1971) for plasma samples ($mEq.l^{-1}$), and a hot 1N nitric acid tissue digest for skeletal and cardiac muscle and liver samples ($mM.kg^{-1}$). B.D.H. (atomic absorption grade) standards were used to establish linear calibration curves, and Versatol "A" was employed as an internal standard for plasma electrolytes. High, medium and zero range standards were used after every five samples to monitor possible instrument drift.

(C). Liver Glycogen Determinations: Liver glycogen was estimated using a modification of the sulfuric-phenol technique of Montgomery (1956). This colourmetric determination (Hitachi 124 Double-beam Spectrophotometer) yielded results as % glycogen by weight.

(D). Water Content and Distribution and Estimation of Approximate Cellular Cation Concentrations: Tissue water levels ($kg.kg^{-1}$) for skeletal muscle and liver were determined by comparing dry tissue (dried at 105°C for 48 hours) to wet tissue weight. Estimates of extracellular phase volume were based upon the chloride ($H_2O^{ecs}_{Cl^-}$) and chloride-potassium ($H_2O^{ecs}_{Cl^-K^+}$) methods of Manery (1954) and Conway (1957) respectively.

Estimates using these two techniques are open to valid criticisms. Chloride and sodium space-based determinations assume confinement of Cl^- and Na^+ to the extracellular compartment (Manery, 1954). The chloride-potassium approach of Conway (1957) assumes distribution of the pair between the cellular and extra-

1. See Appendix page 116.

cellular phases as required by the Donnan relationship. Of the substances currently used to estimate extracellular volume ^{14}C-PEG 4000 provides the most reliable estimates (Hickman, 1972; Schmidt-Nielsen et al., 1972). Recent work has shown a very close relationship between ^{14}C-PEG 4000 and chloride-potassium spaces in skeletal muscle of trout and an acceptable relationship between the two in liver (Mearow and Houston, 1976, unpublished observation). Accordingly estimates of extracellular volume and cellular ion levels have been based upon use of the chloride-potassium approach of Conway (1957). (See Appendix for Equations).

Data Analysis: All data was analyzed with Wang 700 or 2200 computers using statistical packages supplied.

(A) Weight Analysis: Linear regressions of each parameter against weight were performed and their relationship expressed as $y = a + bx$.

(B) Analysis of Variance (ANOVA): Single line ANOVA were performed to determine the significance of temperature and photoperiodic influences on each parameter.

RESULTS

Plasma Electrolyte Variations in Relation to Temperature*: Sodium and chloride, the major plasma electrolytes (Fig. 4) tended to be lower at higher temperatures under both sets of photoperiod conditions. The observed differences were significant ($p < 0.01$) in all cases except chloride under 18L/6D photoperiod. The differences encountered, however, were not large, representing changes of only 6 and 7.5% for plasma sodium and chloride respectively.

Conversely, the less abundant plasma electrolytes (potassium, calcium and magnesium) varied directly with temperature, increasing significantly ($p < 0.01$) in all cases except that of plasma magnesium in 6L/18D animals. In general, these changes represented changes of 20 to 50%.

Since sodium and chloride are the most abundant plasma electrolytes, their inverse temperature relationship was also reflected in an inverse relationship for both the sum of all plasma ions (which approximates osmolarity) and to a lesser extent the sum of all cations. The "apparent anion deficit" (which is defined as the Σcations - chloride and suggests the magnitude of changes in other anions such as bicarbonate and/or phosphate) was relatively thermostable except at the lowest temperature, where it decreased significantly in winter animals.

As indicated in TABLE I, there were significant ($p < 0.05$ or better) temperature-related alterations in 80% of the plasma ions.

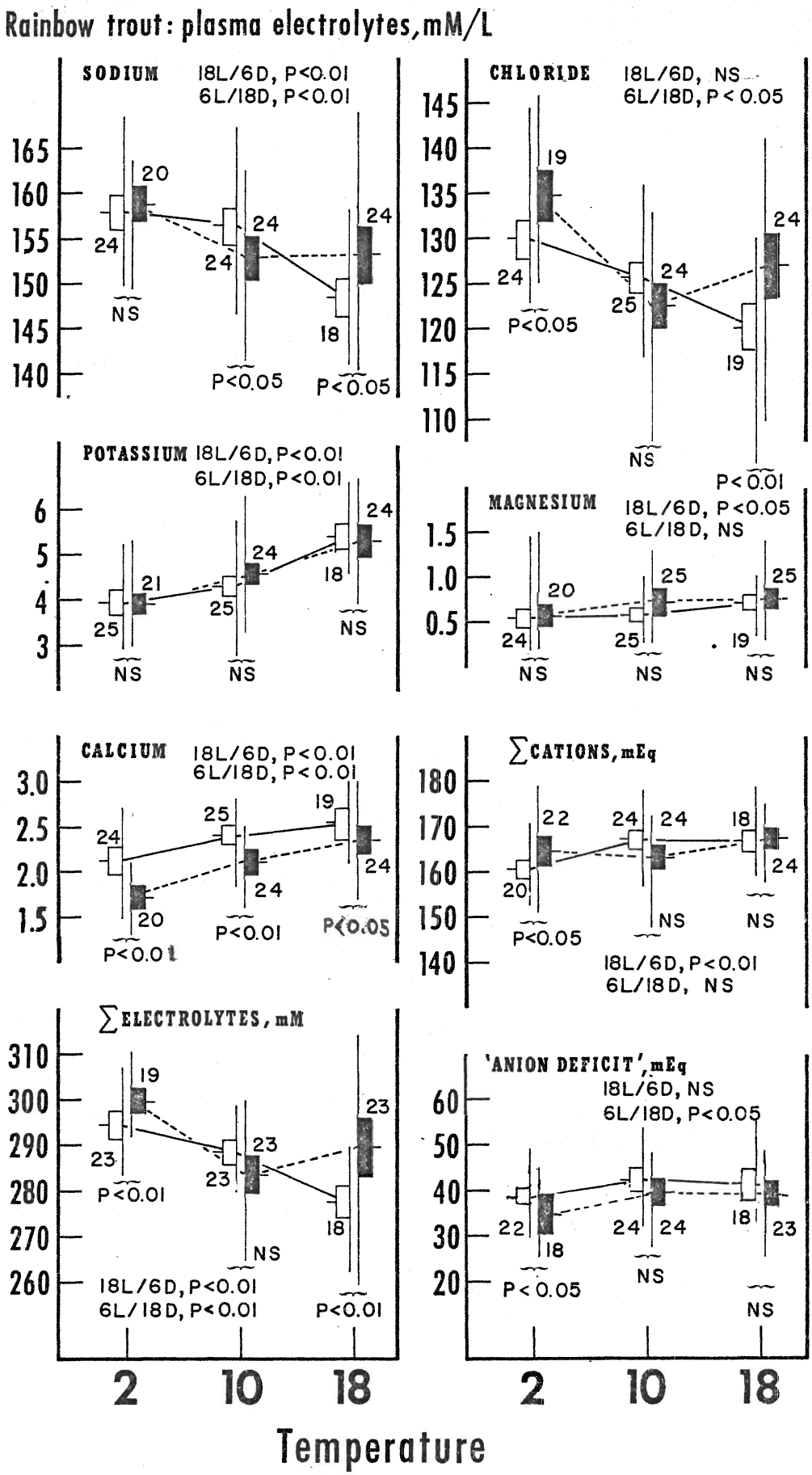
*Plasma electrolyte levels are summarized in TABLE I

TABLE I Plasma electrolyte levels ($mM.l^{-1}$), total plasma ion and cation levels and apparent anion deficit levels.

	PHOTOPERIOD	ACCLIMATION TEMPERATURE °C			Sig.***
		2	10	18	
Sodium	18L/6D	157.8±1.0 (24)* [±1.99]	156.4±1.0 (24) [±2.05]	148.4±1.1 (18) [±2.24]	0.01
	6L/18D	158.8±0.9 (20) [±1.78]	152.9±1.1 (24) [±2.36]	153.2±1.5 (24) [±3.06]	0.01
$mM.l^{-1}$	Sig.**	N.S.+	0.05	0.05	
Chloride	18L/6D	129.9±1.0 (24) [±2.03]	125.6±0.9 (25) [±1.89]	120.2±1.3 (19) [±2.65]	N.S.
	6L/18D	134.7±1.4 (19) [±2.90]	122.5±1.3 (24) [±2.69]	127.0±1.7 (24) [±3.58]	0.01
$mM.l^{-1}$	Sig.	0.01	N.S.	0.01	
Potassium	18L/6D	4.0±0.13 (25) [±0.27]	4.3±0.11 (25) [±0.23]	5.4±0.14 (18) [±0.3]	0.01
	6L/18D	3.9±0.13 (21) [±0.27]	4.6±0.11 (24) [±0.23]	5.3±0.17 (24) [±0.35]	0.01
$mM.l^{-1}$	Sig.	N.S.	N.S.	N.S.	
Calcium	18L/6D	2.1±0.07 (24) [±0.14]	2.4±0.05 (25) [±0.10]	2.5±0.06 (19) [±0.13]	0.01
	6L/18D	1.7±0.06 (20) [±0.13]	2.1±0.06 (24) [±0.12]	2.4±0.07 (24) [±0.14]	0.01
$mM.l^{-1}$	Sig.	0.01	0.01	0.05	
Magnesium	18L/6D	0.5±0.04 (24) [±0.08]	0.6±0.03 (24) [±0.06]	0.7±0.03 (19) [±0.06]	0.01
	6L/18D	0.6±0.06 (20) [±0.13]	0.7±0.07 (25) [±0.15]	0.8±0.05 (25) [±0.10]	N.S.
$mM.l^{-1}$	Sig.	N.S.	N.S.	N.S.	
$\sum$ions	18L/6D	294.2±1.5 (23) [±3.07]	288.3±1.3 (23) [±2.63]	277.5±1.6 (18) [±3.44]	0.01
	6L/18D	299.8±1.3 (19) [±2.75]	283.3±1.9 (23) [±3.98]	289.2±3.0 (23) [±6.24]	0.01
$mM.l^{-1}$	Sig.	0.01	N.S.	0.01	
$\sum$cations	18L/6D	166.9±1.1 (22) [±2.31]	167.0±1.0 (24) [±2.13]	160.6±1.0 (18) [±2.17]	0.01
	6L/18D	167.4±1.0 (20) [±2.11]	163.0±1.2 (24) [±2.52]	164.6±1.5 (24) [±3.15]	N.S.
$mM.l^{-1}$	Sig.	N.S.	0.05	0.05	
Apparent Anion Deficit	18L/6D	38.5±1.0 (22) [±2.08]	42.2±1.2 (23) [±2.43]	40.9±1.8 (18) [±3.71]	N.S.
	6L/18D	34.1±1.8 (18) [±3.74]	39.4±1.3 (23) [±2.54]	38.2±1.3 (23) [±2.74]	0.05
mEq.l	Sig.	0.05	N.S.	N.S.	

* mean ± 1 standard error (sample number) [95% confidence interval]
**level of significance for photoperiod effect at each temperature
***level of significance for temperature effect at each photoperiod
+N.S. not significant at the 0.05 level.

Figure 4. Plasma electrolyte levels, total plasma ion and cation levels and apparent anion deficit levels. Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.


Rainbow trout: plasma electrolytes, mM/L
SODIUM 18L/6D, P<0.01
6L/18D, P<0.01
CHLORIDE 18L/6D, NS
6L/18D, P<0.05
POTASSIUM 18L/6D, P<0.01
6L/18D, P<0.01
MAGNESIUM 18L/6D, P<0.05
6L/18D, NS
CALCIUM 18L/6D, P<0.01
6L/18D, P<0.01
∑CATIONS, mEq
18L/6D, P<0.01
6L/18D, NS
∑ELECTROLYTES, mM
18L/6D, P<0.01
6L/18D, P<0.01
'ANION DEFICIT', mEq
18L/6D, NS
6L/18D, P<0.05
2
10
18
Temperature

Plasma Electrolyte Variation in Relation to Photoperiod:

Photoperiod did not significantly influence plasma levels of magnesium or potassium at any of the acclimation temperatures. In 7 of the 9 remaining cases there were significant photoperiod effects (i.e. for sodium, calcium and chloride). Other plasma parameters such as, the sum of all ions and cations and "apparent" anion deficit were significantly influenced by photoperiod in 5 of the 9 cases. In total 50% of the plasma parameters were significantly affected by photoperiod, while displaying no bias towards any specific temperature.

If consideration is given to conditions in which nominal temperature and photoperiod have been reversed (i.e. 18°C, 6L/18D and 2°C, 18L/6D) in relation to nominal "summer" (18° C, 18L/6D) and "winter" circumstances (2°C, 6L/18D) some evidence of possible photoperiodic compensation for temperature effects is apparent. By comparing the absolute difference between mean values of nominal animals (18°C, 18L/6D - 2°C, 6L/18D) and photoperiod reversed animals (18°C, 6L/18D - 2°; 18L/6D) one can determine whether reversal of the photoperiod compensated for the effect of temperature (i.e. reduced the mean difference between nominal "summer" and nominal "winter" fish). Photoperiod reversal tended to compensate to some degree for temperature effects. Thus comparison between nominal and photoperiod reversed circumstances suggested a substantial reduction in the mean concentration changes for sodium, calcium and chloride. The most notable reductions were in the two major electrolytes, sodium and chloride. These differences decreased from 10.4 to 4.6 and 14.5 to 2.9 $mEq.l^{-1}$ respectively.

In summary, plasma electrolyte levels were significantly influenced by temperature in all but two cases (18L/6D chloride and 6L/18D magnesium). With the exceptions of potassium and magnesium photoperiod differences were also associated with significant variations, with no bias toward any specific acclimation temperature being apparent.

Skeletal Muscle Electrolyte Variation in Relation to Temperature:

Data for skeletal muscle electrolytes is summarized in Fig. 5. Under both "summer" and "winter" photoperiod regimes 80% of the electrolytes displayed significant temperature-related changes; the exceptions being sodium and calcium in "winter" animals. As was the case with the major plasma electrolytes, the absolute differences in skeletal muscle electrolyte concentrations were relatively modest. The principal exception to this generalization was observed in relation to muscle potassium which displayed variations ranging from 12.5% in "winter" fish to 40% in "summer" trout.

Skeletal Muscle Electrolyte Variation in Relation to Photoperiod:

Skeletal muscle electrolyte levels (TABLE II) also exhibited distinct variations in relation to photoperiod. Magnesium levels were higher in "summer" fish at all temperatures, while the converse was true for muscle calcium. As was the case with plasma, sodium and chloride varied inversely with temperature in "summer" fish. The converse relationship existed for muscle chloride in "winter" animals, and potassium in "summer" trout. Potassium levels in "winter" fish displayed a peak level at the intermediate 10^{o}C temperature.

TABLE II Skeletal muscle electrolyte concentrations ($mM.kg^{-1}$)

ELECTROLYTE	PHOTOPERIOD	ACCLIMATION TEMPERATURE °C			Sig.***
		2	10	18	
Sodium	18L/6D	12.8 ± 0.2 (25)* [± 0.50]	11.5 ± 0.2 (25) [± 0.39]	10.9 ± 0.2 (19) [± 0.46]	0.01
	6L/18D	11.8 ± 0.3 (19) [± 0.67]	11.6 ± 0.2 (25) [± 0.41]	12.3 ± 0.2 (24) [± 0.48]	N.S.
$mM.kg^{-1}$	Sig.**	0.05	N.S.+	0.01	
Chloride	18L/6D	9.9 ± 0.2 (24) [± 0.43]	9.5 ± 0.2 (25) [± 0.41]	9.1 ± 0.2 (19) [± 0.36]	0.05
	6L/18D	8.2 ± 0.2 (20) [± 0.42]	8.4 ± 0.2 (25) [± 0.31]	9.1 ± 0.2 (25) [± 0.35]	0.01
$mM.kg^{-1}$	Sig.	0.01	0.01	N.S.	
Potassium	18L/6D	89.7 ± 4.1 (25) [± 8.38]	123.4 ± 4.7 (24) [± 9.62]	128.3 ± 4.2 (18) [± 8.93]	0.01
	6L/18D	109.6 ± 5.2 (20) [± 10.78]	129.2 ± 2.1 (25) [± 4.33]	113.0 ± 5.3 (25) [± 10.94]	0.01
$mM.kg^{-1}$	Sig.	0.01	N.S.	0.05	
Calcium	18L/6D	2.7 ± 0.2 (25) [± 0.31]	2.0 ± 0.1 (25) [± 0.25]	2.7 ± 0.3 (19) [± 0.53]	0.01
	6L/18D	3.5 ± 0.2 (20) [± 0.42]	3.3 ± 0.2 (25) [± 0.39]	3.4 ± 0.2 (24) [± 0.37]	N.S.
$mM.kg^{-1}$	Sig.	0.01	0.01	0.05	
Magnesium	18L/6D	11.3 ± 0.1 (24) [± 0.27]	12.3 ± 0.1 (24) [± 0.14]	12.2 ± 0.1 (19) [± 0.21]	0.01
	6L/18D	10.9 ± 0.2 (19) [± 0.40]	11.9 ± 0.1 (25) [± 0.21]	11.8 ± 0.1 (23) [± 0.23]	0.01
$mM.kg^{-1}$	Sig.	0.05	0.01	0.01	

* mean $\pm$ 1 standard error (sample number) [95% confidence interval]

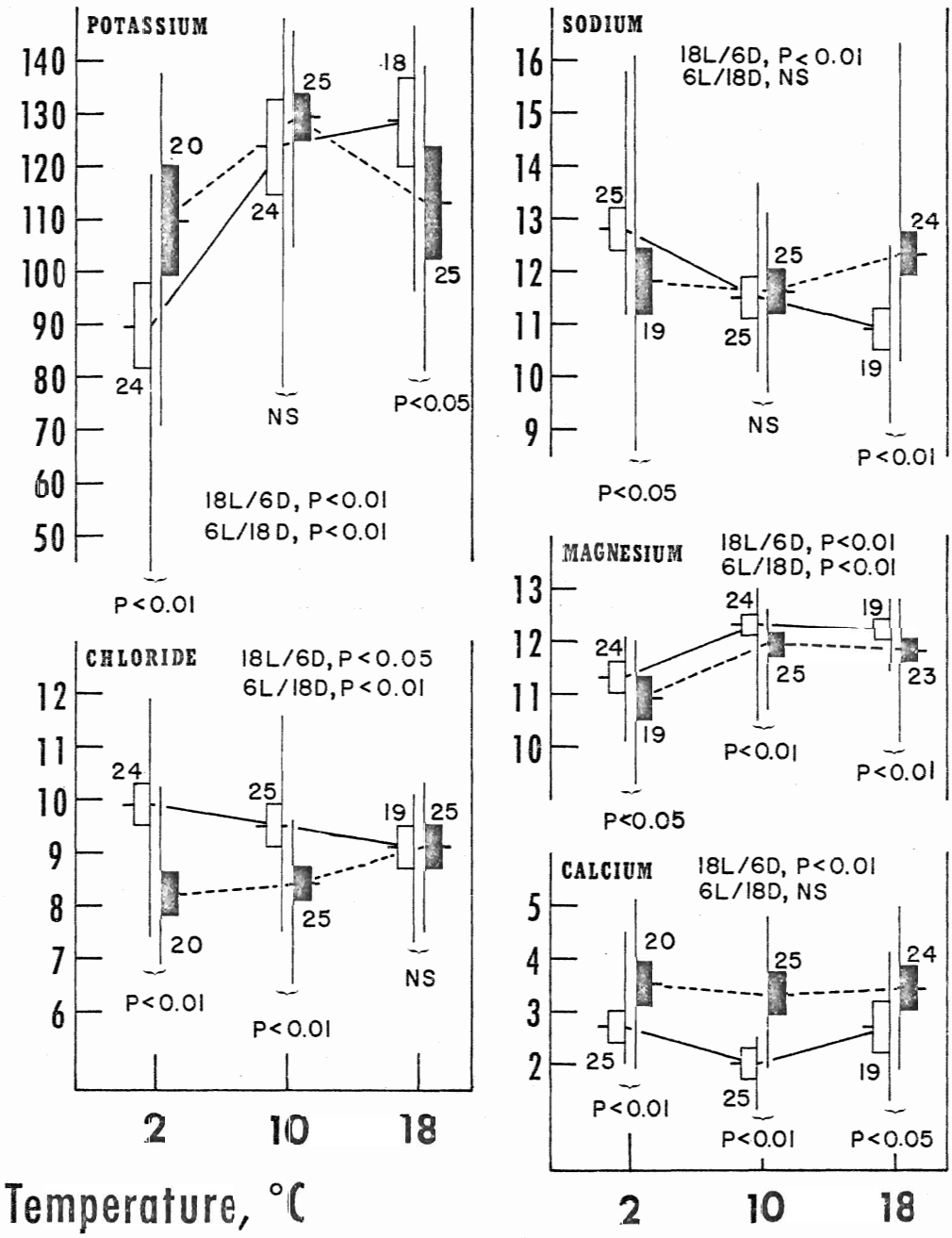
** level of significance for photoperiod effect at each temperature

*** level of significance for temperature effect at each photoperiod

\+ N.S. not significant at the 0.05 level

Figure 5. Skeletal muscle electrolyte concentrations. Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.

Rainbow trout: skeletal muscle electrolytes, mM/kg



Photoperiod reversal, in all cases except potassium reduced the changes in electrolyte concentration associated with temperature acclimation. Significant ($p < 0.05$ or better) photoperiod influences were observed in 80% of the tests, but showed no apparent bias toward any temperature regime.

Liver Tissue Electrolyte Variation in Relation to Temperature:

Data for liver electrolyte concentrations is summarized in Fig. 6 and TABLE III. With the exception of chloride levels in "summer" photoperiod fish, all liver electrolytes were significantly influenced by temperature. Liver sodium levels under both photoperiod regimes and chloride in "winter" photoperiod fish were elevated at higher temperatures. Under "summer" photoperiod conditions liver chloride levels varied inversely with temperature, as did potassium and magnesium under both photoperiod conditions. Calcium was unique in that lower values were observed, under both photoperiod regimes, at the intermediate temperature (10°C). The largest absolute changes occured in sodium, which increased by 40% in "winter" and 18% in "summer" day fish. Chloride levels also increased 19% in "winter" fish.

Liver Tissue Electrolyte Variations in Relation to Photoperiod:

Only 33% of the comparisons made gave evidence of significant photoperiod effects. The most prominent of these was evident in liver chloride which, in "summer" fish, remained thermostable, but displayed a near-linear increase in concentration between 2° and 18°C in "winter" fish. Sodium levels showed significant photoperiod-correlated variations at 2° and 10°C but not at 18°C. Photoperiod reversals compensated for temperature changes in sodium and chloride levels. Potassium, magnesium and calcium did not appear to be significantly influenced by variations in photoperiod regimes.

TABLE III Liver tissue electrolyte concentrations ($mM.kg^{-1}$)

ELECTROLYTE	PHOTOPERIOD	ACCLIMATION TEMPERATURE °C			Sig.***
		2	10	18	
Sodium	18L/6D	29.1 ± 0.9 (25)* [± 1.86]	33.1 ± 0.6 (24) [± 1.24]	34.4 ± 1.2 (19) [± 2.52]	0.01
	6L/18D	25.7 ± 0.6 (21) [± 1.25]	29.7 ± 0.9 (25) [± 1.84]	36.4 ± 0.7 (25) [± 1.49]	0.01
$mM.kg^{-1}$	Sig.**	0.01	0.01	N.S.+	
Chloride	18L/6D	43.3 ± 0.6 (25) [± 1.16]	42.7 ± 0.8 (25) [± 1.67]	41.9 ± 0.9 (19) [± 1.93]	N.S.
	6L/18D	37.3 ± 1.1 (21) [± 2.29]	41.3 ± 0.6 (21) [± 1.31]	45.0 ± 0.8 (23) [± 1.68]	0.01
$mM.kg^{-1}$	Sig.	0.01	N.S.	0.05	
Potassium	18L/6D	108.6 ± 1.1 (25) [± 2.25]	100.8 ± 1.4 (24) [± 2.81]	99.0 ± 3.2 (19) [± 6.72]	0.01
	6L/18D	105.3 ± 1.8 (21) [± 3.75]	101.8 ± 1.2 (25) [± 2.48]	97.5 ± 1.2 (25) [± 2.48]	0.01
$mM.kg^{-1}$	Sig.	N.S.	N.S.	N.S.	
Calcium	18L/6D	0.65 ± 0.07 (25) [± 0.14]	0.51 ± 0.03 (25) [± 0.06]	0.82 ± 0.05 (19) [± 0.11]	0.01
	6L/18D	0.61 ± 0.07 (21) [± 0.15]	0.50 ± 0.05 (25) [± 0.10]	0.94 ± 0.08 (25) [± 0.17]	0.01
$mM.kg^{-1}$	Sig.	N.S.	N.S.	N.S.	
Magnesium	18L/6D	8.1 ± 0.2 (25) [± 0.35]	7.3 ± 0.2 (24) [± 0.43]	7.3 ± 0.15 (19) [± 0.32]	0.01
	6L/18D	8.2 ± 0.2 (21) [± 0.46]	7.3 ± 0.2 (24) [± 0.33]	6.8 ± 0.15 (25) [± 0.32]	0.01
$mM.kg^{-1}$	Sig.	N.S.	N.S.	0.05	

* mean $\pm$ 1 standard error (sample number) [95% confidence interval]

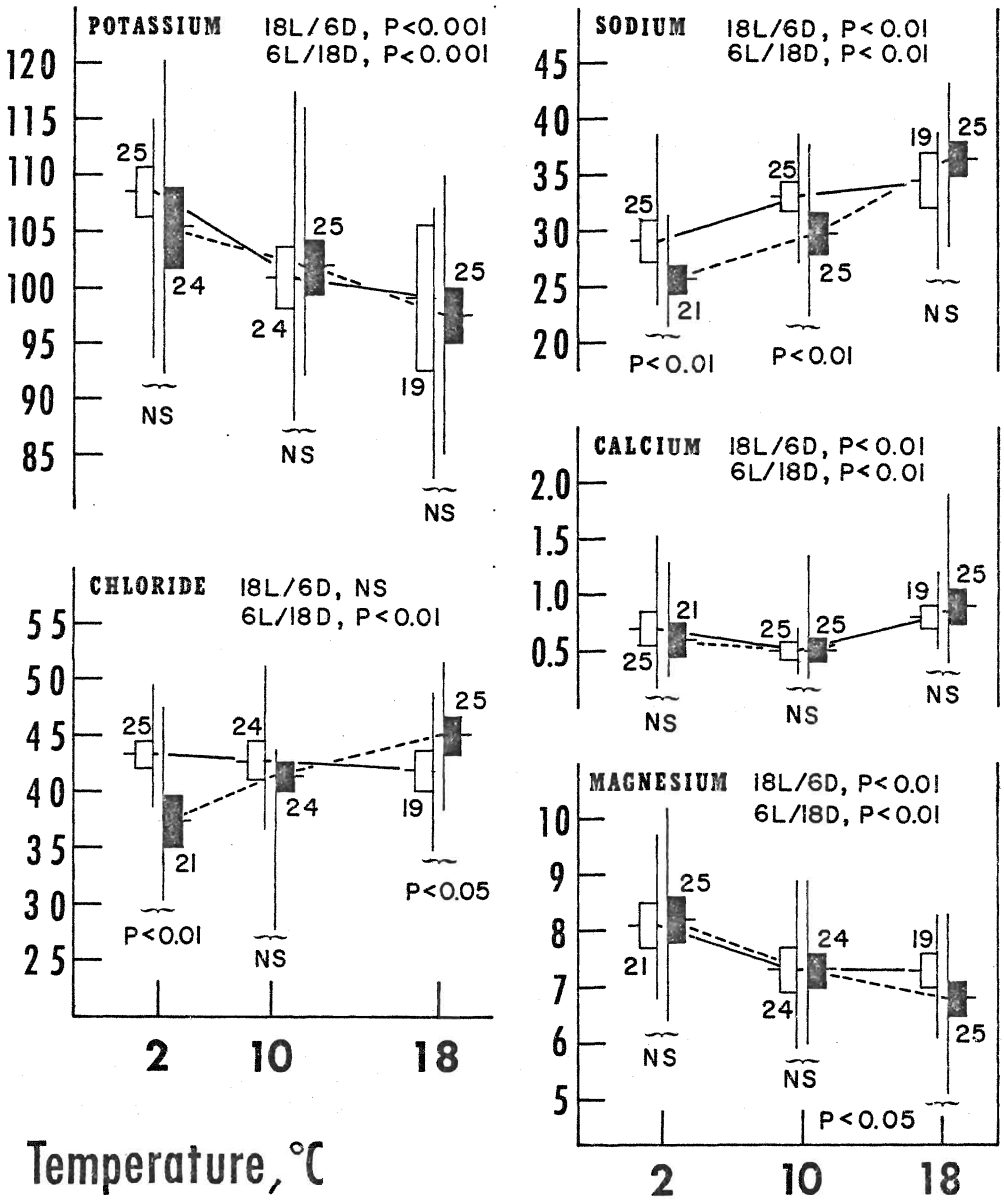
** level of significance for photoperiod effect at each temperature

*** level of significance for temperature effect at each photoperiod

+ N.S. not significant at the 0.05 level.

Figure 6. Liver tissue electrolyte concentrations. Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.

Rainbow trout: liver electrolytes, mM/kg



Although liver electrolytes were extremely thermosensitive (90% of the tests), liver appeared to be the least photosensitive of tissues; only 33% of the analyses showing significant photoperiod variations.

Cardiac Electrolyte Variations in Relation to Temperature:

Observations upon cardiac electrolytes are summarized in Fig. 7 and TABLE IV. No estimates of cardiac chloride were possible due to the small size of the tissue samples ($\leq$ 0.5g). With two exceptions, all cardiac electrolytes were significantly ($p < 0.05$ or better) influenced by alterations in environmental temperature. The two exceptions were calcium levels in fishes held on the 18L/6D regime (which was essentially thermostable) and potassium in animals of the 6L/18D group (which exhibited a non-significant peak at 10°C). Sodium and magnesium varied directly with temperature under both photoperiod regimes. The latter electrolytes were also characterized by the greatest alterations in absolute concentrations observed; 29% increase in sodium for "summer" trout, and 29% and 24% increases in magnesium for "summer" and "winter" fish respectively. Cardiac calcium levels in "winter" trout generally declined with temperature and, as was the case with liver, displayed a non-significant minimum value at 10°C. Potassium in "summer" fish generally increased and displayed a peak level at 10°C.

Cardiac Electrolyte Variation in Relation to Photoperiod:

Cardiac potassium, calcium and magnesium values were higher in "winter" photoperiod fish at all temperatures, whereas sodium levels were higher at all temperatures in "summer" fish. The

TABLE IV Cardiac muscle electrolyte concentrations ($mM.kg^{-1}$)

ELECTROLYTE	PHOTOPERIOD	ACCLIMATION TEMPERATURE °C			Sig.***
		2	10	18	
Sodium	18L/6D	23.8 ± 0.8 (24)* [±1.66]	27.8 ± 0.7 (24) [±1.43]	30.6 ± 1.2 (19) [±2.58]	0.01
	6L/18D	23.5 ± 0.8 (20) [±1.61]	24.7 ± 0.7 (25) [±1.42]	26.8 ± 0.6 (24) [±1.14]	0.01
$mM.kg^{-1}$	Sig.**	N.S.+	0.01	0.01	
Potassium	18L/6D	62.2 ± 0.8 (24) [±1.70]	66.9 ± 0.8 (25) [±1.73]	64.5 ± 0.7 (19) [±1.53]	0.01
	6L/18D	64.7 ± 0.8 (21) [±1.75]	67.5 ± 1.2 (25) [±2.48]	66.2 ± 1.0 (25) [±1.98]	N.S.
$mM.kg^{-1}$	Sig.	0.05	N.S.	N.S.	
Calcium	18L/6D	1.24 ± 0.3 (24) [±0.62]	1.3 ± 0.2 (25) [±0.43]	1.08 ± 0.3 (19) [±0.69]	N.S.
	6L/18D	3.1 ± 0.3 (21) [±0.69]	2.1 ± 0.2 (25) [±0.43]	2.20 ± 0.3 (25) [±0.60]	0.05
$mM.kg^{-1}$	Sig.	0.01	0.01	0.05	
Magnesium	18L/6D	5.11 ± 0.07 (24) [±0.14]	6.2 ± 0.08 (25) [±0.17]	6.6 ± 0.07 (19) [±0.15]	0.01
	6L/18D	5.9 ± 0.23 (21) [±0.48]	6.6 ± 0.17 (25) [±0.35]	7.3 ± 0.19 (25) [±0.39]	0.01
$mM.kg^{-1}$	Sig.	0.01	0.05	0.01	

* mean ± 1 standard error (sample number) [95% confidence interval]

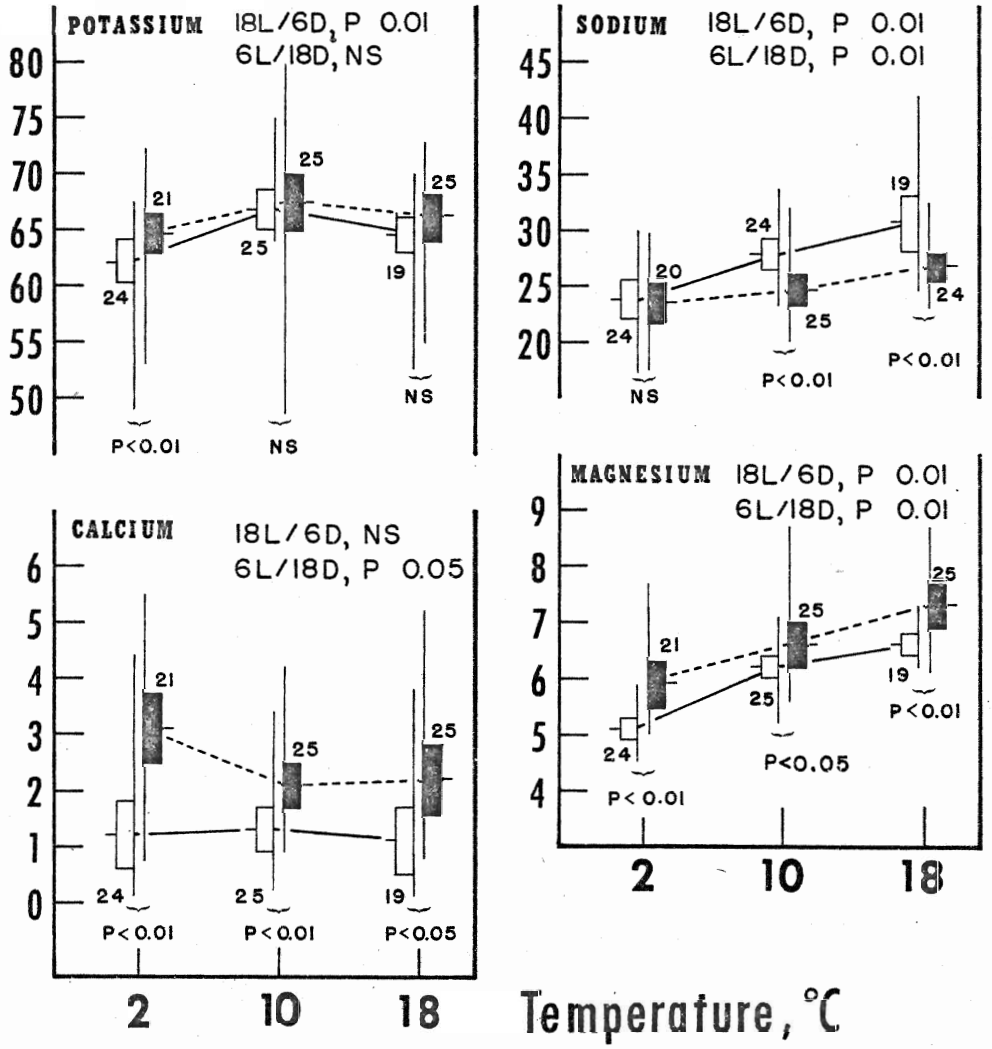
** level of significance for photoperiod effect at each temperature

*** level of significance for temperature effect at each photoperiod

+ N.S. not significant at the 0.05 level.

Figure 7. Cardiac muscle electrolyte concentrations. Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.

Rainbow trout: cardiac muscle electrolytes, mM/kg



most dramatic photoperiod effect in cardiac muscle was, however seen in the case of calcium. In this instance the calcium concentrations in the specimens of the 18L/6D group were thermostable, whereas calcium concentrations in the "winter" group were significantly higher at all temperatures, and most notably so at the two temperature extremes. In only three cases were significant photoperiod effects absent; sodium at 2°C, and potassium at 10° and 18°C. Again, the significant photoperiod effects were not biased by temperature.

Photoperiod reversals tend to produce some compensation for temperature effect especially on calcium and sodium. These reductions amounted to 52 and 60% respectively.

In all, 75% of the analyses of cardiac electrolytes displayed significant temperature, as well as photoperiod-dependent variations.

Water Content and Distribution: Data for skeletal muscle and liver water parameters are listed in TABLE V, Fig. 8(a)(b).

(b) Skeletal Muscle: Water content and distribution in "summer" photoperiod trout were not influenced by temperature. In "winter" fish extracellular volume was also thermostable. In these animals, however, both water content and intracellular phase volumes ($H_2O^{ics}_{Cl^-}$ and $H_2O^{ics}_{Cl^- - K^+}$) were inversely related to temperature ($p < 0.01$). The estimates of these two parameters in "winter" fish were significantly larger than in the 18L/6D fish at the two lowest temperatures, thereby indicating strong photoperiod effect at low temperatures. Although estimates of extracellular space ($H_2O^{ecs}_{Cl^-}$ and $H_2O^{ecs}_{Cl^- - K^+}$) increased at elevated temperatures in "winter" animals, these values were

consistently lower at all temperatures than those of "summer" animals.

Photoperiod variations appear to significantly alter the skeletal muscle water content and distribution in Salmo gairdneri, and especially at lower temperatures. Approximately 90% of the analyses made at 2, and 10°C were significant, whereas none were significant at 18°C. In every case photoperiod reversal had some compensatory influence, ranging in magnitude from 50 to 70%.

In summary, skeletal muscle water parameters appeared to be thermosensitive under "winter" photoperiod conditions, and while under these conditions fish were considerably more sensitive to photoperiod adjustments at lower than higher temperatures.

(b) Liver Tissue: Liver tissue exhibited a pattern almost identical to that of skeletal muscle. The only major discrepancy in thermosensitivity in "summer" trout was seen in the direct relationship which existed between temperature and water content ($p < 0.01$). Again, photoperiodic influence was most apparent at the two lowest temperatures. At these two temperatures 80% of the tests showed significant photoperiod effects, while no significant effects were observed at 18°C.

With the one exception of liver water content, for which there was no compensation, photoperiod reversals prompted compensatory variations similar to those observed in skeletal muscle.

In both liver and skeletal muscle of "winter" fish acclimated to lower temperatures, there appeared to be a strong tendency for water to shift from the extracellular to the intracellular compartment. Conversely, at higher temperatures these animals

TABLE V: Water content and distribution in skeletal muscle and liver tissue.

TISSUE		SKELETAL MUSCLE				LIVER			
Parameter	Photoperiod	ACCLIMATION TEMPERATURE °C				ACCLIMATION TEMPERATURE °C			
		2	10	18	SIG.***	2	10	18	Sig.***
Tissue H_2O	18L/6D	771.3±1.9(25)* [±3.92]	770.7±1.5(25) [±3.33]	773.8±2.2(19) [±4.62]	N.S.	720.7±4.2 (25) [±8.67]	748.1±3.5 (25) [±7.22]	757.1±1.3 (19) [±2.73]	0.01
	6L/18D	784.4±1.6(21) [±3.33]	780.5±1.2(25) [±2.48]	773.7±1.9(25) [±3.92]	0.01	741.6±4.5 (21) [±9.39]	735.9±2.3 (25) [±4.75]	751.0±3.3 (25) [±6.81]	0.01
$g.kg^{-1}$	Sig.**	0.01	0.01	N.S.+		0.01	0.01	N.S.	
$H_2O^{ecs}_{Cl^-}$	18L/6D	69.0±1.6(25) [±3.30]	68.3±1.4(25) [±2.89]	68.0±1.2(19) [±2.52]	N.S.	298.4±5.0(25) [±10.32]	306.5±5.5 (25) [±11.35]	313.8±6.4 (18) [±13.5]	N.S.
	6L/18D	56.1±1.6(21) [±3.34]	61.2±1.2(25) [±2.48]	65.6±1.6(25) [±3.30]	0.01	250.6±7.2 (21) [±15.02]	284.8±7.8 (25) [±16.1]	317.1±6.5 (25) [±13.42]	0.01
ml.kg	Sig.	0.01	0.01	N.S.		0.01	0.05	N.S.	
$H_2O^{ecs}_{Cl^-K^+}$	18L/6D	50.5±2.7(25) [±5.57]	55.8±2.4(25) [±4.95]	50.2±1.9(19) [±3.99]	N.S.	326.5±5.7 (25) [±11.87]	333.8±6.4 (25) [±13.21]	338.5±7.7 (18) [±16.25]	N.S.
	6L/18D	43.7±2.4(20) [±5.02]	47.5±1.5(25) [±3.10]	48.4±2.0(25) [±4.13]	N.S.	270.1±8.2 (21) [±17.11]	308.4±9.1 (25) [±18.78]	343.3±7.6 (25) [±15.69]	0.01
$ml.kg^{-1}$	Sig.	N.S.	0.01	N.S.		0.01	0.05	N.S.	
$H_2O^{ics}_{Cl^-}$	18L/6D	701.9±2.3(24) [±4.66]	702.5±1.4(25) [±2.89]	705.7±2.4(19) [±5.04]	N.S.	422.3±6.6 (25) [±13.62]	441.6±6.7 (25) [±12.83]	443.4±6.5 (18) [±13.72]	N.S.
	6L/18D	725.9±3.4(21) [±7.09]	719.5±1.7(25) [±3.51]	712.1±4.2(25) [±8.67]	0.01	491.1±6.9 (21) [±14.39]	451.0±8.1 (25) [±16.72]	433.8±5.9 (25) [±12.18]	0.01
$ml.kg^{-1}$	Sig.	0.01	0.01	N.S.		0.01	N.S.	N.S.	
$H_2O^{ics}_{Cl^-K^+}$	18L/6D	720.8±2.7(24) [±5.63]	715.0±2.3(25) [±4.75]	723.6±2.7(19) [±5.67]	N.S.	394.0±7.2 (25) [±14.86]	414.3±7.5 (25) [±15.48]	418.7±7.8 (18) [±16.46]	N.S.
	6L/18D	737.7±4.1(20) [±8.58]	733.1±2.0(25) [±4.13]	725.4±2.3(25) [±4.75]	0.05	471.6±7.7 (21) [±16.06]	427.4±9.3 (25) [±19.2]	407.6±6.9 (25) [±14.24]	0.01
$ml.kg^{-1}$	Sig.	0.01	0.01	N.S.		0.01	N.S.	N.S.	

* mean ± 1 standard error (sample number) [95% confidence interval]

** level of significance for photoperiod effect at each temperature

*** level of significance for temperature effect at each photoperiod

+ N.S. not significant at the 0.05 level

Figure 8 (a). Water content and distribution in skeletal muscle and liver tissue (based upon $H_2O^{ics}_{Cl}$). Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.

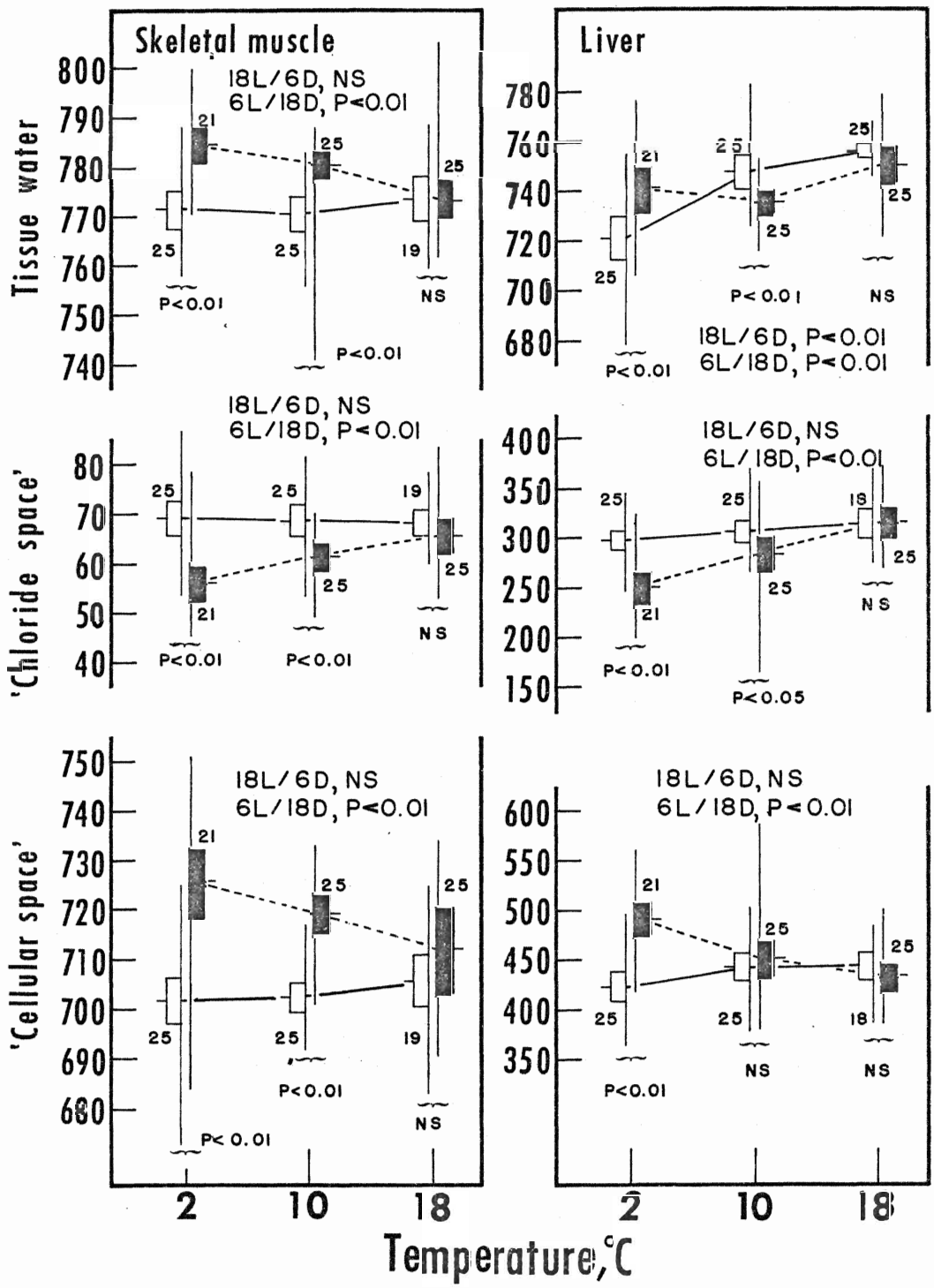
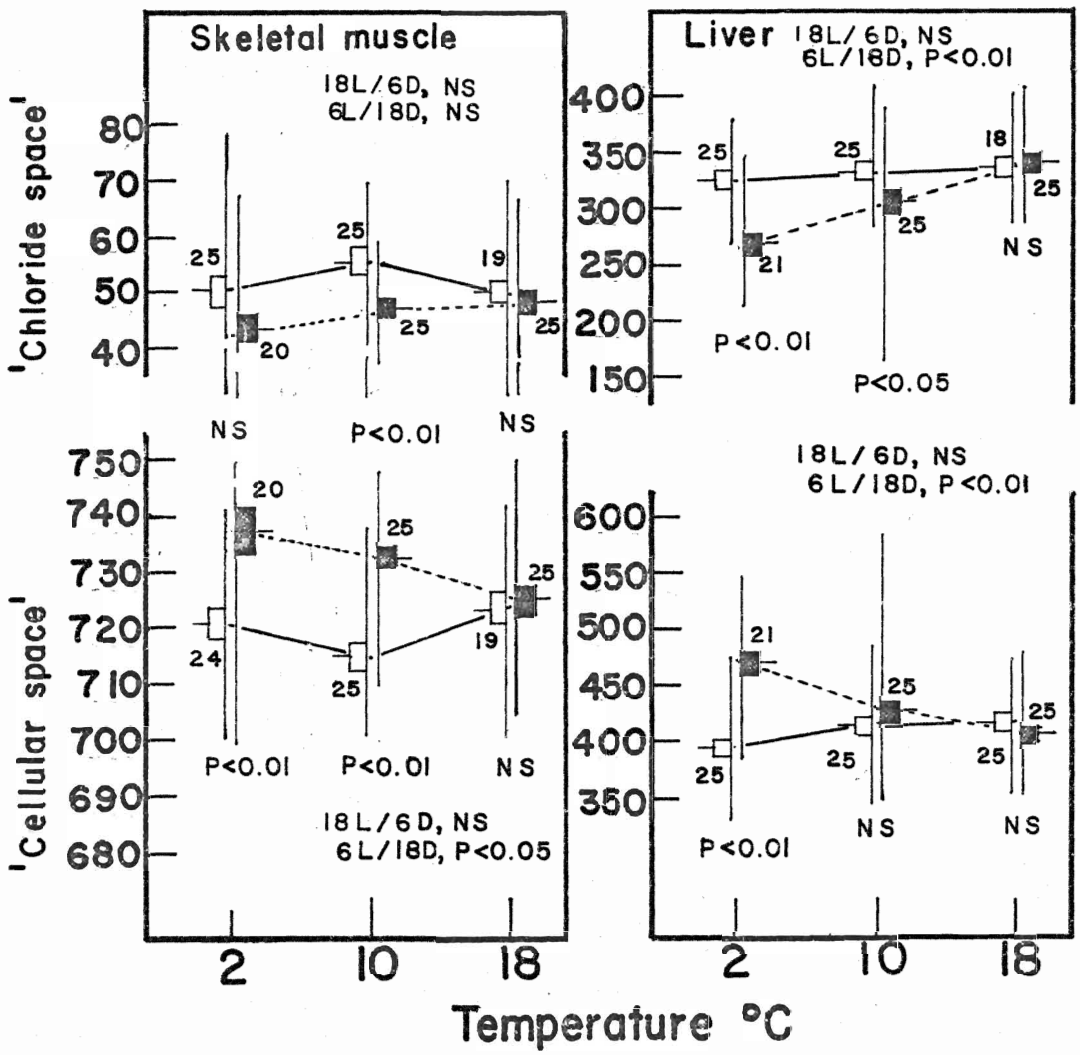

Skeletal muscle
Liver
18L/6D, NS
6L/18D, P<0.01
18L/6D, P<0.01
6L/18D, P<0.01
Tissue water
'Chloride space'
'Cellular space'
P<0.01
P<0.05
NS
2
10
18
Temperature, °C

Figure 8 (b). Water distribution in skeletal muscle and liver tissue (based upon $H_2O^{ics}_{Cl^-\ K^+}$). Horizontal line, the mean of the sample; vertical bar, one standard error (open 18L/6D; closed 6L/18D); vertical line, range of the data.


Skeletal muscle
18L/6D, NS
6L/18D, NS
Liver 18L/6D, NS
6L/18D, P<0.01
'Chloride space'
'Cellular space'
18L/6D, NS
6L/18D, P<0.05
18L/6D, NS
6L/18D, P<0.01
NS
P<0.01
NS
P<0.01
P<0.05
P<0.01
P<0.01
NS
P<0.01
NS
NS
2
10
18
Temperature °C

were characterized by increases in extracellular phase volume. In "summer" trout the extracellular and intracellular volumes in both muscle and liver showed no significant change with temperature. Generally water balance in rainbow trout appears to be quite sensitive to alteration in photoperiod regimes as well as thermal environment.

Cellular Ion Concentrations: Estimates of cellular cation concentrations were based upon use of both $H_2O^{ecs}_{Cl^-}$ and $H_2O^{ecs}_{Cl^- - K^+}$ spaces for liver and skeletal muscle and are summarized in TABLE VI.

(a) Skeletal Muscle: Cellular potassium levels increased with temperature under both light regimes using both extracellular space estimates. Potassium levels were affected by photoperiod only at 2°C. Values at the other two temperatures appeared to be relatively insensitive to photoperiod. Cellular magnesium concentrations increased significantly ($p < 0.01$) over the range 2° to 18°C, with values for the 18L/6D groups being consistently higher than those of the 6L/18D group. "Summer" fish displayed a significant inverse relationship between temperature and cellular sodium levels, with these values being significantly lower in animals held at 10° and 18°C than was the case with "winter" photoperiod animals at the same temperatures. Cellular calcium levels were reduced at 10°C in specimens maintained on both 6L/18D and 18L/6D. The "summer" groups exhibited significant increases in concentrations with temperature. "Winter" day fish, while displaying a similar relationship, were characterized by significantly higher values than "summer" fish at all three test temperatures.

All cellular cation concentrations in the skeletal muscle of "summer" photoperiod rainbow trout were influenced by temperature, whereas only potassium and magnesium were so affected in "winter" fish. Photoperiod thus emerged as a significant factor in 18 of 24 cases with no bias toward any temperature being apparent. Photoperiod reversal only compensated for temperature effects in sodium (an 88% reduction) and magnesium (a 75% reduction).

(b) Liver Tissue: Cellular potassium and magnesium of liver in "summer" trout displayed a significant ($p < 0.01$) inverse relationship with temperature. Values for these ions in "winter" fish, while basically thermostable, were consistently (although not always significantly) lower than corresponding values in "summer" fish at comparable temperatures. The single significant photoperiod effect in these two cations occured at $2^{\circ}C$. In this instance both potassium and magnesium levels in "summer" animals significantly exceeded those of "winter" photoperiod animals.

As had been the case with muscle, calcium levels were again reduced at $10^{\circ}C$. "Winter" fish had equivalent or higher calcium levels at all temperatures, with values at $10^{\circ}C$ being significantly ($p < 0.05$) greater than "summer" fish. Estimates of liver cellular sodium were negligible. This is likely due to an over-estimation of the extracellular phase volume resulting from endogenous chloride, as was the case cited by Lutz (1972) and Mearow and Houston (unpublished). There was no obvious compensation for temperature effects by photoperiod reversal in liver cellular cation levels.

TABLE VI. Estimated cellular cation concentrations ($mM \cdot l^{-1}$ cell water) in skeletal muscle and liver (1- estimates based on $H_2O^{ics}_{cl}$; 2- estimates based on $H_2O^{ics}_{cl-k}$)

Tissue			Skeletal muscle				Liver			
Electrolyte		Photo-period	Acclimation temperature, °C				Acclimation temperature, °C			
			2	10	18	Sig.	2	10	18	Sig.
Sodium	1	18L/8D	3.6±0.4(25)*	2.4±0.5(25)	2.1±0.3(19)	0.05	-	-	-	-
		6L/18D	4.8±0.5(21)	4.0±0.3(25)	4.0±0.4(25)	NS	-	-	-	-
		sig.	NS	0.01	0.01	-	-	-	-	-
	2	18L/6D	7.7±0.6(23)	4.7±0.7(24)	5.2±0.4(18)	0.01	-	-	-	-
		6L/18D	7.5±0.5(19)	6.5±0.3(24)	7.4±0.4(23)	NS	-	-	-	-
		Sig.	0.05	0.05	0.01	-	-	-	-	-
Potassium	1	18L/6D	120±7.4(25)	173±6.8(25)	177±7.3(19)	0.01	256±4.4(25)	234±6.2(25)	221±7.5(19)	0.01
		6L/18D	156±8.2(21)	179±3.0(25)	169±8.0(25)	0.05	213±4.6(21)	224±4.2(25)	222±4.6(25)	NS
		Sig.	0.01	NS	NS	-	0.01	NS	NS	
	2	18L/6D	122±5.8(24)	173±6.7(24)	176±6.2(17)	0.01	273±5.4(25)	243±6.6(24)	238±8.5(17)	0.01
		6L/18D	153±5.5(19)	175±3.1(24)	162±4.7(24)	0.01	222±5.3(21)	238±5.2(24)	237±5.9(24)	NS
		Sig.	0.01	NS	NS	-	0.01	NS	NS	-
Calcium	1	18L/6D	3.5±0.3(25)	2.7±0.2(25)	3.7±0.8(19)	0.01	0.6±0.2(25)	0.1±0.02(25)	0.6±0.1(18)	0.01
		6L/18D	4.9±0.4(21)	4.5±0.3(25)	4.8±0.3(25)	NS	0.6±0.2(21)	0.3±0.1(25)	1.0±0.2(25)	0.01
		Sig.	0.01	0.01	0.05	-	NS	0.05	NS	-
	2	18L/6D	3.6±0.2(23)	2.6±0.2(25)	3.6±0.3(19)	0.01	0.6±0.2(24)	0.1±0.02(25)	0.6±0.1(18)	0.01
		6L/18D	4.6±0.3(19)	4.4±0.3(24)	4.7±0.2(23)	NS	0.6±0.2(20)	0.3±0.1(24)	1.0±0.2(24)	0.01
		Sig.	0.01	0.01	0.05	-	NS	0.05	NS	-
Magnesium	1	18L/6D	16.0±0.2(24)	17.3±0.2(25)	17.2±0.2(19)	0.01	18.7±0.5(25)	16.5±0.6(25)	16.1±0.4(19)	0.01
		6L/18D	15.0±0.4(21)	16.5±0.2(25)	16.6±0.2(25)	0.01	16.4±0.4(21)	15.5±0.6(25)	15.1±0.4(25)	NS
		Sig.	0.05	0.01	NS	-	0.01	NS	NS	-
	2	18L/6D	15.7±0.2(22)	17.2±0.1(24)	16.8±0.2(19)	0.01	20.5±0.5(24)	17.4±0.7(24)	17.1±0.4(18)	0.01
		6L/18D	14.8±0.3(17)	16.2±0.2(25)	16.2±0.2(23)	0.01	17.1±0.5(20)	16.8±0.5(24)	16.1±0.4(25)	NS
		Sig.	0.05	0.01	0.05	-	0.01	NS	NS	-

*mean ± 1 S.E.M. (N)

In summary, liver cellular cations closely followed the pattern observed in skeletal muscle, in that the cations of "winter" animals were generally thermostable, while those of "summer" fish were thermosensitive. These values however do resemble liver electrolyte values in that only 39% of the cases tested yielded any significant photosensitivity and these were strongly biased toward the lowest temperature($2^{o}C$).

Miscellaneous Results:

(a) Hematocrit (TABLE VII). Hematocrit values in rainbow trout increased significantly under both photoperiods. The hematocrit values of "summer" trout although appearing to be larger were statistically the same as those of the "winter" stock of trout. The increases in hematocrit for both photoperiods were in excess of 30% over the range 2^{o} to $18^{o}C$. Photoperiod reversal yielded a 36% compensation for temperature effects.

(b) Liver Glycogen (TABLE VII). Values for liver glycogen were thermostable in "summer" trout. "Winter" animals, however, were characterized by higher glycogen levels than "summer" at each comparable temperature and displayed a maximum level at $10^{o}C$, which was significantly above glycogen levels observed at 2^{o} and $18^{o}C$ in this group. Compensation by photoperiod reversal was 52% for liver glycogen.

(c) Weight-Specific Variations in Electrolyte Levels: Correlation coefficients for weight specific variations of the various tissue electrolytes are summarized in TABLE VIII.

Of the 114 linear regressions calculated for the relationship of weight against various electrolytes, a surprisingly large number (18) showed a significant weight correlation. This corresponds to 16% of the total, and is well above the number which

TABLE VII Liver glycogen levels (% glycogen by weight) hematocrit values (% packed cell volume)

PARAMETER	PHOTOPERIOD	ACCLIMATION TEMPERATURE °C			Sig.***
		2	10	18	
Liver Glycogen (% Glycogen by weight)	18L/6D	1.3 ± 0.7 (23)* [± 1.49]	1.02 ± 0.2 (23) [± 0.44]	0.6 ± 0.3 (19) [± 0.55]	N.S.
	6L/18D	4.4 ± 0.6 (19) [± 1.28]	5.1 ± 0.5 (22) [± 1.14]	3.1 ± 0.5 (25) [± 1.05]	0.05
	Sig.**	0.01	0.01	0.01	
Hematocrit (% packed cell volume)	18L/6D	34.0 ± 0.9 (25) [± 1.73]	35.8 ± 0.8 (25) [± 1.73]	44.1 ± 1.3 (19) [± 2.87]	0.01
	6L/18D	32.1 ± 0.8 (21) [± 1.71]	34.6 ± 0.7 (25) [± 1.37]	41.7 ± 1.0 (25) [± 2.10]	0.01
	Sig.	N.S.+	N.S.	N.S.	

* mean $\pm$ 1 standard error (sample number) [95% confidence interval]

** level of significance for photoperiod effect at each temperature

*** level of significance for temperature effect at each photoperiod

\+ N.S. not significant at the 0.05 level.

might be expected to occur by chance in a sample of this size. The number of positive and negative weight correlations was equal, while the general distribution of significant weight-specific correlations was biased toward higher temperatures and longer photoperiods. Calcium and potassium showed the largest number of weight correlations (7 each).

The following general observations regarding weight-specific variations in electrolytes were immediately obvious. The two plasma electrolytes whose sum total essentially constitute total plasma osmolarity (sodium and chloride) were not significantly correlated with weight. However, potassium the major skeletal muscle electrolyte was positively correlated with weight, and significantly so in 5 of the 6 cases. Plasma potassium in "summer" fish, on the other hand, displayed a significant negative correlation with weight at 10° and 18°C.

Calcium showed significant inverse relationships with weight in 50% of the tests on skeletal muscle and liver. Two thirds of these occured at 18°C. No bias toward photoperiod was observed.

In general, (i.e. considering significant as well as non-significant correlations) potassium was negatively correlated with weight in plasma and conversely positively correlated in skeletal muscle. The exact opposite relationship exists for both divalent cations, with this negative relationship of calcium in skeletal muscle also applying to liver and cardiac muscle.

Reference to photoperiod reversal in relation to weight specific variations in electrolyte levels, brings to light an interesting possibility. Considering the sign (i.e. the nature, either negative or positive) of the correlation between weight

TABLE VIII. Weight specific variations in plasma, skeletal muscle, liver tissue and cardiac muscle electrolyte levels as indicated by correlation coefficients. Bracketted numbers refer to sample size, asterisks indicate correlation coefficients significant at the 0.05 level or better.

ELECTRO-LYTE	TISSUE / PHOTO-PERIOD	PLASMA ACCLIMATION TEMPERATURE °C 2	10	18	SKELETAL MUSCLE ACCLIMATION TEMPERATURE °C 2	10	18	LIVER ACCLIMATION TEMPERATURE °C 2	10	18	CARDIAC MUSCLE ACCLIMATION TEMPERATURE °C 2	10	18
Sodium	18L/6D	-0.398(24)	+0.028(24)	+0.142(18)	-0.337(24)	-0.195(25)	0.000(19)	+0.122(25)	-0.274(24)	+0.654(19)*	+0.182(24)	-0.282(24)	-0.286(19)
	6L/18D	+0.058(20)	+0.227(24)	+0.364(24)	+0.265(19)	+0.081(25)	+0.019(24)	-0.103(21)	-0.267(25)	+0.140(25)	+0.281(24)	-0.077(25)	+0.111(24)
Chloride	18L/6D	+0.095(24)	-0.173(25)	+0.165(19)	-0.394(24)	+0.280(25)	+0.202(19)	+0.066(25)	-0.207(25)	-0.381(19)	-	-	-
	6L/18D	+0.266(19)	-0.056(25)	-0.012(24)	+0.342(20)	-0.113(25)	-0.158(25)	+0.118(21)	-0.282(21)	-0.001(23)	-	-	-
Potassium	18L/6D	-0.273(25)	-0.405(25)*	-0.518(18)*	+0.536(25)*	+0.487(24)*	+0.047(18)	-0.110(25)	-0.031(24)	+0.128(19)	+0.369(24)	-0.331(25)	+0.046(19)
	6L/18D	-0.349(21)	-0.270(24)	+0.061(24)	+0.488(20)*	+0.406(25)*	+0.637(25)*	+0.158(21)	-0.098(25)	+0.174(25)	+0.007(21)	+0.008(25)	-0.143(25)
Calcium	18L/6D	+0.137(24)	-0.175(25)	+0.136(19)	-0.361(25)	-0.619(25)*	-0.549(19)*	-0.296(25)	-0.371(25)	-0.468(19)*	-0.081(24)	-0.024(25)	+0.052(19)
	6L/18D	+0.205(20)	+0.092(24)	+0.530(24)*	-0.110(20)	-0.250(25)	-0.545(24)*	-0.543(21)*	-0.237(25)	-0.554(25)*	-0.020(21)	-0.257(25)	-0.130(25)
Magnesium	18L/6D	-0.256(24)	+0.063(25)	+0.005(19)	-0.127(24)	-0.222(24)	-0.034(19)	-0.147(25)	+0.466(24)*	+0.220(19)	+0.554(24)*	-0.244(25)	+0.074(19)
	6L/18D	-0.308(20)	+0.173(25)	+0.163(25)	-0.429(19)	-0.417(25)*	-0.319(23)	+0.069(21)	-0.261(24)	-0.028(25)	+0.184(21)	-0.197(25)	-0.111(25)

and the 5 electrolytes from the 4 tissue sources (a total of 19 cases) the following general observation was made. In 13 of the 19 cases (68%) the nominal "summer" day (18°C, 18L/6D) and "winter" day (2°C, 6L/18D) conditions yielded correlations in the same direction. However, under photoperiod reversed conditions only 10 have the same sign. Of the 13 original nominal cases of the same sign only 7 remained unchanged, while only 3 of the 6 of opposite sign remained so. It is possible that, under nominal conditions weight could affect the different electrolytes in the same way moreso than it does under photoperiod reversed conditions. Consequently one might speculate that weight specific-variations of electrolyte levels may be dependent upon the intimate "nominal" relationship between temperature and photoperiod.

(d) Weight-Specific Variations in Water Content and Distribution:

Data summarizations of linear regressions of water against weight parameters are given in TABLE IX. As indicated by the asterisks in TABLE IX, 20% of the cases studied yielded significant weight dependent correlations. Again, the significant correlations were biased toward warmer temperatures and longer photoperiods.

(i) Skeletal Muscle Water Parameters: In "summer" trout at 10°C there was a significant positive correlation of $H_2O^{ecs}_{Cl^-\text{-}K^+}$ with weight. This was accompanied by a significant negative correlation of $H_2O^{ics}_{Cl^-\text{-}K}$ and $H_2O^{ics}_{Cl^-}$ with weight at the two highest temperatures. Therefore, "summer" fish appear to show a weight dependent shift of water

from the intracellular to the extracellular phase at higher temperatures. The other two significant weight correlations were with tissue water under nominal "summer" and "winter" conditions; both of which were negative to the same degree.

The only other consistent, although non-significant weight dependent patterns observable were $H_2O^{ecs}_{Cl^-\text{-}K^+}$, which was generally positive and $H_2O^{ics}_{Cl^-\text{-}K^+}$ which was being generally negative in "summer" trout. Bearing this in mind, and recalling that plasma potassium values were negatively correlated with weight and skeletal muscle potassium was positively correlated with weight one could postulate a weight dependent dilution effect in plasma, and a concentrating effect in tissue as monitored by relative potassium levels.

(ii) Liver Tissue Water Parameters: Again, in "summer" fish there was a similar, but opposite relationship between weight and movement of water. The weight-dependent shift of water appeared to be from the extra- to the intracellular phase. Of all of the significant weight-dependent effects, 80% occured under nominal "summer" conditions. These were for estimates of extracellular phase (negative) and intracellular phase (positive).

Considering the effect of photoperiod reversal on both skeletal muscle and liver tissue water parameters, 8 of 10 nominal conditions had the same sign, while only 3 of these 9 remained the same under photoperiod reversals. This tends to support earlier speculation that weight-specific variations may depend upon a close "nominal" relationship between temperature and photoperiod.

TABLE IX. Weight-specific variation in skeletal muscle and liver water content and distribution as indicated by correlation coefficients. Bracketted numbers refer to sample size; asterisks indicate correlation coefficients significant at the 0.05 level or better.

Parameter	Photo-period	Acclimation temperature					
		2°C	10°C	18°C	2°C	10°C	18°C
		Skeletal muscle			Liver		
tH_2O	18L/6D	+0.066(25)	-0.204(25)	-0.642(19)*	+0.412(25)*	-0.340(25)	+0.157(18)
	6L/18D	-0.660(21)*	+0.220(25)	-0.244(25)	-0.016(21)	-0.202(25)	-0.166(25)
$H_2O^{ecs}_{Cl}$	18L/6D	-0.329(25)	+0.339(25)	+0.119(19)	-0.011(25)	-0.188(25)	-0.544(18)*
	6L/18D	+0.185(21)	-0.107(25)	-0.237(25)	-0.109(21)	-0.202(25)	+0.074(25)
$H_2O^{ecs}_{Cl-K}$	18L/6D	+0.249(25)	+0.517(25)*	+0.028(19)	-0.014(25)	-0.166(25)	-0.544(18)*
	6L/18D	+0.231(20)	-0.157(25)	+0.049(25)	-0.092(21)	-0.210(25)	+0.079(25)
$H_2O^{ics}_{Cl}$	18L/6D	+0.271(24)	-0.579(25)*	-0.645(19)*	+0.271(25)	-0.024(25)	+0.532(18)*
	6L/18D	-0.123(21)	+0.218(25)	-0.077(25)	+0.105(21)	+0.133(25)	-0.175(25)
$H_2O^{ics}_{Cl-K}$	18L/6D	-0.206(24)	-0.657(25)*	-0.533(19)*	+0.251(25)	+0.117(25)	+0.566(18)*
	6L/18D	-0.126(20)	+0.245(25)	-0.244(25)	-0.195(21)	+0.199(25)	-0.168(25)

DISCUSSION

The discussion will be broken down into two basic components: (a) the specific effects of temperature and photoperiod (b) the general effects of temperature and photoperiod. Firstly the specific data will be considered in relation to accepted modes of electrolyte recruitment and the overall effects of temperature and photoperiod on each of the various parameters measured in this paper. In doing this, reference to the percentage of significant effects of both temperature and photoperiod on each of the parameters will act as a guideline to indicate the relative impact of these two environmental factors. Secondarily, the data will be considered in a more general nature which will reflect the influence of temperature and photoperiod on cellular metabolism. The primary consideration will point out specific variations caused by temperature and photoperiodic acclimation, whereas, the secondary consideration will elucidate the overall effect of these individual changes on general metabolism.

The Specific Effects of Temperature and Photoperiod: The stresses placed upon the osmo-ionoregulatory process of teleosts acclimated to higher temperatures are largely due to the augmentation of respiratory activity required to meet increased metabolic oxygen demands and the reduced availability of oxygen. Under these circumstances the teleost has two potentially effective compensatory mechanisms: (1) enhancement of blood-oxygen carrying

capacity (e.g. through increasing total hemoglobin modulators) and/or (2) increasing the activity of the branchial counter-current exchanger system (e.g. through elevation of ventilation flow and rate, increase in gill perfusion and effective exchange area, decrease in diffusion pathlength and increase in cardiac output). In the present study hematocrit values increased by 29% over the range of temperature from 2°C to 18°C under both photoperiods. Although this does augment the oxygen carrying capacity, it also increases the viscosity of the blood. This in turn increases the level of cardiac work assuming that vascular resistance remains constant. Since more cardiac work is required, this response is not purely compensatory in nature. As discussed in the Review of Literature, increased oxygen demand resulting from increased temperatures is primarily satisfied by increases in the various constituents of the branchial counter-current exchanger system (Randall, 1968; Randall et al., 1967; Stevens and Randall, 1967(a); (b); Woods and Randall, 1973 (a); (b)) and secondarily satisfied through alterations of one or more components of the oxygen carrying system (Black et al., 1966; Houston and DeWilde, 1968; 1969; Grigg, 1969; Houston et al., 1968; 1970; Houston, 1973; Houston et al., 1976). However, in so satisfying oxygen requirements, fish at higher temperatures are inevitably subject to increased branchial electrolyte loss and endoosmosis if alterations in branchial permeability do not occur. Associated with increases

in water uptake are compensatory increases in glomerulr filtration and urinary flow rates (Isaia, 1972; Motais and Isaia, 1972; Houston, 1973). These, in effect, increase renal electrolyte loss (Fromm, 1968; Isaia, 1972). In order to maintain water-electrolyte balance at elevated temperatures, the obvious utilitarian responses would be: (1) reduction in electrolyte efflux through changes in branchial permeability, (2) increased branchial electrolyte recruitment, (3) increased urinary flow and glomerular filtration rates and (4) increased renal reabsorption of electrolytes. The literature also provides good evidence of endocrine involvement in the above responses.

Branchial electrolyte recruitment involves both heteroionic exchange diffusion and active transport mechanisms. In fresh-water teleosts it is generally accepted that Na^+ is brought into the cell by exchange diffusion for either H^+ or NH_4^+ (both of which are dependent upon cellular carbonic anhydrase activity). Once inside the cell Na^+ appears to be pumped into the blood (at least at higher temperatures) in exchange for K^+ which is brought back into the cell from the blood via a serosal (Na^+-K^+)-ATPase (Maetz, 1971; 1974; Kerstetter and and Kirschner, 1972). Chloride, the other major electrolyte, appears to be brought into the cell via exchange diffusion for HCO_3^- or by active transport via (HCO_3^-)-ATPase (Kerstetter and Kirschner, 1972). It is then restored to the plasma by

some as of yet undefined serosal Cl^- pump mechanism. Recent work by McCarty and Houston (1977) found blood carbonic anhydrase activities to increase with temperature while gill tissue levels showed modest decreased at higher temperatures in rainbow trout, thus, providing potential exchange ions for Cl^- and Na^+.

Branchial microsomal (Na^+-K^+)-ATPase activities increased with temperature in goldfish (Murphy and Houston, 1974) and in rainbow trout (McCarty and Houston, 1977). In the latter study McCarty found: (1) kidney levels of (Na^+-K^+)-ATPase to increase with temperature and (2) gill and kidney levels of (HCO_3^-)-ATPase to increase with temperature as well. Thermal acclimation of freshwater fishes is also accompanied by decreases in plasma bicarbonate and increases in plasma ammonia and hydrogen ion levels (Maetz, 1972; Rahn and Baumgardner, 1972; Powers, 1974) which along with the findings of McCarty and Houston (1977), favour increased exchange uptake of sodium and chloride at higher temperatures.

In the present study, the major electrolytes, although displaying a large degree of temperature sensitivity (i.e. significant temperature related changes were found in 11 of the 14 tests performed) changed very little in the absolute sense when trout were exposed to 2^{o}, 10^{o} and $18^{o}C$. The changes in Na^+ and Cl^- due to temperature averaged 13.6 and 8.9 $mEq \cdot l^{-1}$. The relative changes in all plasma electrolytes was between 5.5

and 8.6% over the temperature range of 2° to 18°C. This confirms the ability of trout to maintain ion balance over a wide range of temperatures. One can assume that compensatory adjustments did occur, especially in the area of active transport enzymes as found by McCarty and Houston (1977).

The following interpretation of specific results must be classified as speculative, being based upon accepted modes of electrolyte recruitment and endocrine involvement. Consideration will be given to the effect of temperature over the range of 2°C to 18°C rather than between the two temperature ranges of 2° to 10°C and 10° to 18°C. In comparing the two photoperiod regimes one must bear in mind the fact that at any given temperature, the strain on both "summer" and "winter" fish as a result of temperature, is approximately equal, yet, the animals on the long (18L/6D) photoperiod have activity periods that are three times longer than the "winter" animals. This in itself would place additional physiological strain on the animals which must be considered in comparing these two groups.

In the present study, plasma Na^+ and Cl^- levels decreased some 5.7 and 7.5% in"summer" fish and 3.7 and 5.7% in "winter" fish respectively over the range of temperatures from 2° to 18°C. Skeletal muscle levels of Na^+ and Cl^- dropped some 14.8% and 8.1% in "summer" animals but showed and increase of 4.8 and 10.9%

in "winter" animals. It is important to recall at this point that these values are the net result of extensive depletion (anticipated at higher temperatures) and enhanced branchial recruitment (also anticipated at higher temperatures). Plasma K^+ levels rose 35% under both photoperiods, whereas skeletal muscle concentarions rose 43% in "summer" fish and only 13% in "winter" animals over the range of 2^{o} to $18^{o}C$. Literature has provided evidence that many of the active components referred to in the Maetz model may have been responsible for these observations. Recently erythrocyte carbonic anhydrase activity in rainbow trout has been found to increase over the same temperature range (Smeda and McCarty, unpublished data), while gill levels of the same enzyme remained relatively constant (McCarty and Houston, 1977). Randall and Cameron (1973) found the plasma HCO_3^- levels in trout to decrease with temperature as was the case with apparent anion deficit (which is thought to reflect HCO_3^- levels) in plasma of this study. These results imply that at elevated temperatures more HCO_3^- is being produced and cleared via the gills. This would provide the necessary reactants within the branchial "chloride" cell for amplification of sodium and chloride uptake at higher temperatures. McCarty and Houston (1977) also found that gill and kidney levels of (HCO_3^-)-ATPase (for active recruitment of chloride) and (Na^+-K^+)ATPase (for active recruitment of sodium into the blood and potassium back into the cell) to increase at

elevated temperatures in rainbow trout. Increases in the activity of these enzymes could have accounted for the relative stability of sodium and chloride.

The following is a proposed explanation of the observed changes in sodium, chloride and potassium in plasma and skeletal muscle of rainbow trout under both photoperiods between 2° and 18°C. The major assumption to be made in this proposal is that the skeletal muscle, being most removed from direct environmental influence would act as a "sink" for electrolytes and respond to relative increases or decreases in plasma electrolyte levels. As environmental water temperatures increase, the stress in terms of satisfaction of increased oxygen demand in the face of decreased oxygen availability increases. In order to satisfy this basic requirement, the fish will increase its ventilation rate and volume and subsequently increase its efflux of Na^+ and Cl^- but not K^+ (it being relatively impermeable at the mucosal border). This anticipated response was reflected in the observed decrease in plasma Na^+ and Cl^- (ranging from 3.7 to 7.5% under both photoperiods). Based upon the intial assumption one would then anticipate subsequent decreases in skeletal muscle levels of Na^+ and Cl^-. However, only in "summer" day fish was this the case (decreases of 14% and 8.1% for Na^+ and Cl^- respectively) The absolute values for "winter" animals rose by some 4.8% and 10.9% for Na^+ and Cl^- respectively. This unexpected increase could be

explained if one considers water movement in "summer" and "winter" animals. The "summer" trout showed no influence of temperature upon total skeletal muscle water levels, extracellular space or intracellular space. However, in "winter" stock, there was a decrease in total tissue water and an apparent shift of water from the intra-cellular space to the extra-cellular space. Although the skeletal muscle Na^+ and Cl^- values showed an increase in "winter" fish, when cellular water levels were taken into account, as is shown in cellular ion concentration of Na^+ and Cl^-, in skeletal muscle, the "winter" animals also displayed a decrease of some 16% over the range of temperatures from 2° to 18°C. Thus supporting the proposal that skeletal muscle may act as a "sink" for electrolytes and respond to changes in plasma values. The differences in sodium and chloride observed between "summer" and "winter" animals at 18°C (that being, higher levels in "winter" fish for estimates of plasma values) could possibly be explained by the fact that the activity period of "winter" fish being substantially less, places less of a strain upon the fish by decreasing its basic oxygen requirement which would in effect reduce net electrolyte efflux.

Carrying the same argument further one can also consider the results observed for plasma and skeletal muscle potassium levels. The major point to be considered in this context is if absolute values of plasma K^+ rose by some 35% in both "summer" and

"winter" fish, why did the skeletal muscle levels in "summer" fish rise 43% as compared to a mere 13% in "winter" fish. There are several factors that should be considered in discussing the observed changes in K^+ levels. Firstly, in the case of potassium, movement is restricted to that between the plasma and muscle since branchial loss of K^+ is negligible. Also, one must recognize the large concentration gradient that exists between the cell and the plasma. This gradient favours movement of potassium from the cell into the plasma. In this study, not only did the plasma K^+ levels increase some 35%, but the hematocrit (%PCV) also increased some 29% under both photoperiods over the range of 2^{o} to $18^{o}C$. This substantially increases the whole blood K^+ concentration. As mentioned earlier there was no substanial change in water parameters in "summer" trout and the increases in plasma potassium were reflected in similar increases in skeletal muscle levels and cellular ion concentration of potassium. In order to maintain cellular K^+ levels there exists a (Na^+-K^+)-ATPase system which pumps K^+ back into the cell against its concentration gradient. If one assumes that the rate of K^+ diffusion into the plasma is finite and that (Na^+-K^+)-ATPase would be functioning at an elevated rate at $18^{o}C$ for a larger percentage of each day in "summer" fish (i.e. 18 hours compared to 6 hours that the fish are actively swimming) then a subsequently

larger build up of cellular potassium would be anticipated in "summer" trout. If this is the case, then, perhaps the amount of potassium in the plasma of "winter" fish which is not being taken up into the cell could be excreted as suggested in figure 3 (page 42).

Presently, it is impossible to ascertain whether these postulated responses did occur and the animal does respond purely to relative changes in plasma concentration levels. There is a strong likelihood that these initial changes also stimulated endocrine responses which could have mediated the observed results. Certainly, the possibility of the latter must be considered.

Endocrine Involvement: The following consideration of endocrine involvement in the compensatory adjustments of teleosts to thermal acclimation and photoperiod regulation must also be classified as speculative (Fig. 2).

Prolactin functions primarily to reduce branchial Na^+ efflux through restriction of passive permeability (Ball, 1969). Plasma and pituitary levels of prolactin vary directly in relation with temperature (McKeown and Peter, 1976) and photoperiod (Lam and Hoar, 1967; Ball, 1969; Ogawa, 1970; McKeown and Peter, 1976). Stimulation of prolactin secretion by temperature and

photoperiod may have contributed to the relatively limited effect of temperature on plasma and skeletal muscle levels of Na^+ by reducing branchial efflux. In concert with this, cortisol administration has been found to increase Na^+influx in adrenalectomized eels (Chan et al., 1969); increase (Na^+-K^+)-ATPase in intact eels (Epstein et al., 1907) and to restore lost (Na^+-K^+)-ATPase in hypophysectomized eels (Butler, 1973). Photoperiod regulation of cortisol activity as well, cannot be ruled out in light of the fact that (1) Bailey and Fenwick (1975) found the photosensitive pineal gland at least partially controlled activity within the hypothalamus (which controls the adrenal secretion of cortisol) and (2) the finding of Potts and Fleming (1970) that prolactin injection caused an increase in plasma cortisol levels.

The possibility of a functional renin-angiontensin-aldosterone system in teleosts cannot be eliminated (Fig. 3). Aldosterone would account for increased renal regulation Na^+ and K^+ as well as excess water. Its regulation may fall under the jurisdiction of the corpuscle of Stannius (Ogawa, 1968; Chan et al., 1969; Bailey and Fenwick, 1975), which could be activated via the photosensitive pineal.

Calcium regulation (Fig. 9) appears to fall under the control of several endocrine systems. The corpuscles of Stannius are accepted as being responsible for maintaining a hypocalcemic plasma in freshwater teleosts (Ogawa, 1969; Chan et al., 1969; Leloup-Hately, 1970; Pang, 1971; Butler, 1972; Bailey and Fenwick 1975 (a)). The ultimobranchial gland was also ascribed as having a "calcitonin-like" hypocalcemic function in freshwater teleosts

Figure 9. Pathway indicating possible endocrine involvement in the maintenance of a hypocalcemic plasma in teleost fishes and the influence of hypercalcemia upon kidney function.

↑ PHOTOPERIOD
??????????????????????????????????
? ↑ PINEAL STIMULATION OF ?
? ?
? HYPOTHALAMUS ?
??????????????????????????????????
↑ PLASMA CALCIUM
↑ PLASMA CALCIUM
↑ ULTIMOBRANCHIAL ACTIVITY
↑ CORPUSCLE OF STANNIUS ACTIVITY
↑ BLOOD PRESSURE
↓ GFR
↓ PLASMA CALCIUM
HYPOCALCEMIC PLASMA
SEE FIGURE 3

(Copp et al., 1968). Rasquin and Rosenbloom (1954) found the ultimobranchial gland to display a hyperplasic as well as hypertrophic response to total darkness. Pang (1971) may have linked the function of the corpuscle of Stannius to the function of the ultimobranchial, when stanniectomized fish displayed results similar to those observed by Rasquin and Rosenbloom (1954). Bailey and Fenwick (1975) postulated that the increased branchial uptake of calcium promoted by the hormone prolactin (Ball, 1969; Pang, 1973) may be related to the photosensitive pineal stimulation of the pituitary. In the present study plasma calcium levels displayed significant increases under both photoperiods over the range of 2° to 18° C. Skeletal muscle calcium was thermostable in "winter" fish, while "summer" fish displayed a signifcantly lower value at 10° C.

Administration of cortisol and prolactin tends to reduce the ill-effects (decreased urine flow and glomerular filtration rates, and increased urinary sodium concentrations) associated with adrenalectomy and hypophysectomy (Stanley and Fleming, 1967(a); Chan et al., 1969; Lam, 1969; Potts and Fleming, 1970; Butler, 1973; MacFarlane, 1974). This would indicate a possible involvement of cortisol and prolactin in renal compensation for water loading at higher temperatures as was found by Lam and Leatherland (1969). "Summer" trout in this study had very stable skeletal muscle water levels, whereas "winter" trout had significantly higher tissue water levels at 2° and 10° C. This possibly indicates a photoperiod stimulation of prolactin levels in "summer" fish at all temperatures increased renal excretion of water, and decreased excessive branchial water loading and thereby reduced endoosmosis and the

effects of endoosmosis at higher temperatures.

Thus, the observations of water and electrolyte status of trout made in this study, may be attributable in part at least to photoperiodic and thermal influences upon various endocrine systems. However, proper evaluation of endocrine involvement in the thermal and photoperidd acclimatory processes in fish will require considerable qualitative and quantitative examination of their photoperiod and temperature sensitivities.

Liver Glycogen in Response to Increased Temperature and Photoperiod: Liver glycogen levels in "winter" fish decreased significantly ($p < 0.05$) with temperature and were consistently higher than in "summer" fish at all three temperatures. Since liver glycogen is believed to be broken down to blood sugar by epinephrine (a blood catecholamine secreted by the adrenal medulla in response to increased physiological stress) it appears that (1) fish under both photoperiod conditions are under more stress at higher temperatures (therefore consume more glycogen) (2) fish under "summer" photoperiod conditions are under more physiological stress than "winter" fish at all temperatures (presumably due to increased activity periods under these light conditions, 18L/6D). These results further support the previous speculation that perhaps fish under similar temperature regimes, but longer photoperiods, are under additional physiological strain.

The General Effects of Temperature and Photoperiod: Another area which lends itself to speculation is the overall impact that the cellular ionic environment has upon metabolic control. Bygrave (1967) in reviewing this topic suggests that the ionic environment can and does control certain metabolic activities especially phosphotransferase reactions. Recognizing for example that Mg^{++} and K^{+} stimulate pyruvate kinase activity, whereas Ca^{++} and Na^{+} inhibit this reaction, he proposed that this key enzyme in glycolysis can be regulated by the relative concentrations of these four ions. Based upon the present study, one could in a very simplistic manner, consider the following hypothetical situation: (a) there is a ratio of stimulatory (Mg^{++} and K^{+}) to inhibitory (Ca^{++} and Na^{+}) electrolytes which controls glucose metabolism most efficiently. (b) In striving for this optimal efficiency, an animal worries less about variations in specific electrolytes in specific tissues but concerns itself more with maintenance of this optimal or near optimal ratio of stimulatory to inhibitory electrolytes within each tissue. (c) Perhaps a "master" gland (i.e. the hypothalamus), being in a position to view the body or tissues as a whole functions via the endocrine system by adjusting its activity to suit whatever stress the animal encounters.

Accepting this one could go on to determine the ratio of stimulatory to inhibitory electrolytes in the various tissues at each temperature and under both photoperiods. The hypothesis

could be that under various strenuous circumstances the animal would adjust to maintain a certain ratio if possible. Therefore one might anticipate a somewhat constant ratio under different stress situations (in this specific case, increased temperature and extended photoperiod).

To test this hypothesis a crude approach was taken, namely to determine the ratio between the sum of the mean values for Mg^{++} and K^{+} and the sum of the mean values for Ca^{++} and Na^{+} for each tissue at all three temperatures under both photoperiods regimes (Table X). The results of this admittedly crude test reveal some rather interesting possibilities. The most striking factor is that no matter what the specific changes were in each electrolyte of each tissue the ratio of stimulatory to inhibitory electrolytes is remarkably similar. In most cases the pattern of the ratio for "summer" and "winter" day fish were at least similar if not identical. Moreover, when you extend the study further to calculate the ratio for all four tissues in total, a striking similarity is also present. Thus, the possibility that the animal (trout) does place a priority upon a strict regulation of glycolysis via the electrolytes that stimulate and inhibit rather than individual electrolyte balance could exist.

Intrinsic Weight Factor: Very few authors have considered

TABLE X The ratio of stimulatory to inhibitory electrolyte levels at 2, 10, and 18°C under "summer" (18L/6D) and "winter" (6L/18D) photoperiod conditions.

	2°C	10°C	18°C
PLASMA			
18L/6D	.028*	.031	.040
6L/18D	.028	.033	.039
SKELETAL MUSCLE			
18L/6D	6.52	10.05	10.33
6L/18D	7.88	9.47	7.95
LIVER			
18L/6D	3.92	3.22	3.02
6L/18D	4.31	3.61	2.79
CARDIAC MUSCLE			
18L/6D	2.68	2.51	2.24
6L/18D	2.65	2.76	2.53
PLASMA + SKELETAL MUSCLE + LIVER + CARDIAC MUSCLE			
18L/6D	1.25	1.37	1.40
6L/18D	1.35	1.45	1.29

* $$\frac{\text{Mean } Mg^{++} \text{ concentration} + \text{mean } K^{+} \text{ concentration}}{\text{Mean } Ca^{++} \text{ concentration} + \text{mean } Na^{+} \text{ concentration}}$$

weight dependent variations in water-electrolyte status in teleost fishes. This study provides evidence that this factor is deserving of much closer scrutiny. Sixteen percent (some 18 out of 114) regressions of various electrolyte levels vs. weight yielded significant (at least $p < 0.05$) weight-dependent variations. In this study there was a slight bias toward "summer" fish, and a strong bias towards elevated acclimation temperatures. Weight was a significant factor in 20% (12 out of 60) of the water content and distribution vs. weight analyses. In this case there was a strong bias towards "summer" animals (11:1) and towards higher acclimation temperatures (2:3:7). In skeletal muscle there was an apparent weight-dependent shift of water from the cellular to the extracellular phase, whereas the reverse relationship held true for liver tissue.

Therefore, while spurious correlations may have been observed, it is obvious that specimen size does have a significant influence upon some aspects of water-electrolyte balance in rainbow trout.

CONCLUSION

Rainbow trout, Salmo gairdneri, acclimated to temperatures of 2, 10 and 18°C under two photoperiods(18h light:6h dark and 6h light:18h dark)can invoke compensatory processes necessary to meet elevated oxygen demands, while maintaining water-electrolyte balance. However, in so maintaining general overall water-electrolyte balance there were specific cases of significant temperature and photoperiod influences. Significant ($p < 0.05$ or better) temperature-related variations occured in 70% (68 out of 96) of the analyses performed, showing no bias towards either photoperiod. Significant ($p < 0.05$ or better) photoperiod-related variations occured in 57% (82 out of 144) analyses performed at common temperatures. Photoperiod-related variations did however show a bias towards lower temperatures (35:27:20) for 2:10:18°C respectively. There was a noticeable absence of photoperiod influence upon water content and distribution in 18°C fish.

The interaction of temperature and photoperiod upon water electrolyte balance was quite evident in this study. Photoperiod reversal resulted in a reduction in the differences observed between nominal "summer" and "winter" animals in 60% of the cases examined. The degree of compensation varied from 10% to as high as 96% in the case of apparent anion deficit.

Significant weight dependent variations in water-electrolyte levels occured in some 17% of 174 analyses, a frequency that exceeds the number expected by chance alone.

Implications as to the possible role played by various endocrine systems in compensatory adjustments to increased temperature and prolonged photoperiod have been suggested but

not verified.

What one can conclude from this study is that temperature and photoperiod do affect water-electrolyte balance in rainbow trout. Whether this effect triggered a response in each tissue for each electrolyte or whether one factor caused a cascading effect in which case the other factors responded to its change, is impossible to determine. The explanations given in the discussion give two possibilities namely (a) that one system (i.e. skeletal muscle) may respond to changes in plasma which were induced by alterations in temperature and/or photoperiod, or, (b) that the animal will attempt to regulate the cellular metabolism by stabalizing the stimulatory/inhibitory electrolyte levels if some factor like temperature and/or photoperiod induce changes in existing electrolyte levels.

It is also quite likely that endocrine systems are involved in maintaining water electrolyte balance especially under circumstances of increased temperature and prolonged photoperiod.

One can safely assume that any research into the influence of temperature upon electrolyte balance must seriously consider the light (photoperiod) conditions. Otherwise, the observations that are being attributed to variations in temperature may in fact be due to photoperiod (or vice versa). One must also pay closer attention to the influence of body weight upon

water-electrolyte status.

This study has considered a small aspect of water-electrolyte balance in rainbow trout. It does however, provide the first comprehensive examination showing that two environmental factors, temperature and photoperiod, do have a strong influence upon water-electrolyte balance both individually and cooperatively. Future research steming from this might include: (a) how the rainbow trout recruits electrolytes at the branchial and renal levels when acclimated to higher temperatures and/or prolonged photoperiods, (b) in vivo and in vitro studies of the influence of ionic ratios upon glycolysis, (c) the influence of temperature upon specific endocrine systems, (d) the influence of photoperiod upon specific endocrine systems, and, (e) the influence of endocrine systems upon water-electrolyte balance in rainbow trout.

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APPENDIX

DERIVED EQUATIONS

Estimates of Extracellular space

1. $$H_2O^{ecs}_{Cl} = 0.9 \frac{t\ Cl^-}{pl\ Cl^-} \cdot 1000 = ml.kg^{-1}$$

2. $$H_2O^{ecs}_{Cl\text{-}K} = 1000 \times \frac{(tCl^-)(tK^+)-(tH_2O)(iCl^-)(iK^+)}{(tCl^-)(iK^+)+(iCl^-)(tK^+)-2(tH_2O)(iCl^-)(iK^+)}$$

$= ml.kg^{-1}$

t = tissue

pl = plasma

$iCl^- = 0.977 \times pl\ Cl^-$

$iK^+ = 1.06 \times pl\ K^+$

$tH_2O = kg.kg^{-1}$

Estimates of Intra-Cellular Space

3. $H_2O^{ics}_{Cl^-} = tH_2O - H_2O^{ecs}_{Cl^-} = ml.kg^{-1}$

4. $H_2O^{ics}_{Cl^-}{}_{K^+} = tH_2O - H_2O^{ecs}_{Cl^-\text{-}K^+} = ml.kg^{-1}$

Estimates of Cellular Ion Concentrations

$$[\ ]_{Cl^-} = tx - \frac{plx.r.H_2O^{ecs}_{Cl}}{H_2O^{ics}_{Cl}} \times 1000 = mM.l^{-1}\ cell\ H_2O$$

To obtain cellular concentrations based on Cl^--K^+ space substitute $H_2O^{ecs}_{Cl^-\text{-}K^+}$ and $H_2O^{ics}_{Cl^-\text{-}K^+}$

r = Donnan Factor

$Na^+ = 0.94$

$K^+ = 0.94$

$Cl^- = 1.02$

$Ca^{++} = 0.714$

$Mg^{++} = 0.867$

x = electrolyte being considered

Belehradek equation:

$$- \text{Log } \dot{V}O_2 = K_o + K_i \cdot \text{Log } T$$

- $\dot{V}O_2$ = Oxygen Consumption (ml O_2/g)
- T = Temperature in $^{\circ}$C
- K_o = Basal metabolic rate
- K_i = Van't Hoff Q_{10}

Plasma Chloride

- 0.05 ml of thoroughly mixed plasma sample
- 4.0 ml acetic acid reagent (0.1 N nitric + 10% acetic acid)
- 4 drops of gelatin indicator
- titrate at high rate (Cotlove Chloridometer)
- standard used-Labtrol
- reagent: to 900 ml H_2O add 6.4 ml concentrated nitric acid and 100 ml glacial acetic acid
- calculation of unknown concentration

$$\frac{(\text{Std. conc.})(\text{Unknown time} - \text{Blank time})}{(\text{Std. time} - \text{Blank time})}$$

Tissue Chloride

- weighed, dried tissue sample
- sonicate in 5.0 ml 1.5 N NaOH
- marble placed upon tube
- heat in boiling water bath for 30 minutes
- cool and transfer to 25 ml volumetric flask and dilute to volume with H_2O
- transfer 10.0 ml to test tube
- add 1.0 ml 20% zinc sulfate $\cdot$ 7 H_2O in 2 N nitric acid
- shake and let sit for 1 hour
- centrifuge for 20 minutes at 2000 rpm
- add 2.0 ml of supernatent into titration vials (duplicates)
- add 0.1 ml of fresh alkaline perborate
- leave for 16 to 24 hours at room temperature

- add three drops of gelatin indicator (should turn blue)
- add 0.5 ml of 1.3 N nitric acid in 50% acetic acid (should turn pink or yellow)
- titrate at low rate
- standard used-5 ml of 4 micro. eq./ml NaCl in 1.5 N NaOH or Labrol may be used in NaOH
- Reagent- 1.5 N NaOH, 1.3 N nitric acid in 50% acetic acid, 2 N nitric in 20% zinc sulfate $\cdot$ 7 H_2O, alkaline perborate $\cdot$ 7 H_2O in 5 ml 6N NaOH
- calculation of micro eq. Cl /g wet tissue

$$\frac{(20\ \mu\text{ eq. std.})(\text{Unknown time} - \text{Blank time})}{(\text{Std. time} - \text{Blank time})(\text{g tissue})}$$

Tissue Sodium, Potassium, Magnesium and Calcium

- tissue samples were digested in 5.0 ml hot nitric acid (1N) until totally digested
- 5 ml was diluted to 100 ml with H O contianing 0.25% strontium chloride(prevents spectral interference of K^+ on Ca^{++}
- this 1:20 dilution placed the samples within the linear portion of the standard curves of the Perkin Elmer 403 atomic absorption spectrophotometer

RAINBOW TROUT 18 L/6 D, 2°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	96	M	153.8	3.05	.21	0.91	-----	15.8	89.9	10.1	2.28	----	29.6	115.1	9.7	0.63	42.1	0.02	21.9	58.5	5.2	0.78
02	107	F	149.8	2.94	---	2.32	144.5	11.2	86.1	12.0	2.15	10.3	23.2	111.8	8.2	0.43	41.4	1.50	21.1	64.0	5.2	0.63
03	79	M	162.6	4.37	.87	2.52	136.3	13.1	63.0	11.8	4.68	8.9	30.4	102.4	8.0	0.60	42.2	----	----	----	---	----
04	93	F	-----	4.93	.54	2.70	137.4	11.8	99.0	12.0	2.40	11.4	31.7	111.9	8.2	0.69	44.6	0.03	21.2	66.1	5.9	4.43
05	109	F	157.7	3.15	.54	2.39	128.9	11.7	93.7	11.5	2.82	10.4	38.7	106.5	8.6	0.51	41.2	0.08	34.0	66.3	5.7	0.50
06	91	M	153.8	3.93	.33	2.02	126.6	11.9	98.9	12.0	2.09	10.8	33.6	112.6	9.3	0.45	46.0	0.10	17.3	63.6	5.2	0.83
07	80	M	154.7	3.28	.33	1.49	132.1	13.4	55.3	10.8	2.31	11.0	32.6	119.3	9.5	0.67	39.9	0.02	22.0	61.6	5.2	0.42
08	94	F	153.3	4.11	.50	2.19	127.6	11.9	106.6	11.7	2.16	9.8	28.6	110.9	8.0	1.42	43.4	0.19	29.4	65.2	5.1	0.59
09	63	M	161.2	4.39	.96	2.44	132.7	13.0	76.7	11.8	3.81	10.1	33.8	105.3	7.5	0.66	46.5	0.02	20.7	62.6	4.7	0.43
10	110	F	152.0	3.77	.58	2.49	125.0	10.7	106.6	11.4	2.27	7.4	24.2	104.4	7.6	0.88	42.6	0.03	20.7	60.9	5.2	2.91
11	77	F	158.2	3.79	.62	2.27	123.8	11.7	72.2	11.0	2.71	9.2	26.4	111.6	6.8	1.52	43.0	1.30	19.3	66.0	5.1	3.46
12	101	F	158.2	4.11	.75	2.22	126.4	14.8	87.2	11.8	3.06	9.7	35.5	109.2	8.6	0.77	47.4	0.05	20.6	65.6	5.2	3.10
13	71	F	168.3	4.08	.66	2.44	128.2	12.8	97.2	11.7	4.53	9.0	27.8	110.9	7.5	1.34	42.1	0.16	28.4	54.1	4.7	4.06
14	81	F	163.9	4.18	.62	2.29	133.6	10.8	50.6	----	2.63	10.6	27.2	115.0	8.0	1.15	42.9	0.11	21.2	66.5	5.0	3.90
15	79	F	156.4	4.26	.37	1.97	128.8	13.7	80.8	11.7	2.96	9.7	26.7	109.2	8.8	0.83	38.5	0.72	29.4	67.3	5.8	0.74
16	100	F	158.2	3.36	.37	2.27	129.2	13.2	92.1	11.6	2.05	9.5	31.0	105.1	7.8	0.39	42.7	0.98	25.1	62.2	5.1	0.18
17	101	M	156.8	5.06	.54	1.66	132.0	14.6	118.5	10.5	3.31	9.1	17.7	93.7	6.0	0.17	38.3	16.2	26.2	61.9	5.1	0.07
18	95	M	159.5	3.90	.37	1.97	125.0	12.5	116.1	11.4	1.98	9.5	27.7	99.5	8.3	0.30	42.3	1.30	23.7	66.3	5.0	0.05
19	106	F	155.1	4.24	.54	1.94	129.5	13.0	86.9	10.6	2.78	9.5	26.2	113.9	8.6	0.38	49.6	0.94	28.5	60.6	5.4	0.20
20	66	M	157.3	5.01	.46	2.14	128.8	14.1	43.2	10.4	2.25	11.4	27.4	105.3	6.9	0.41	43.4	----	20.3	58.1	4.4	0.03
21	85	M	154.2	3.31	.58	1.84	125.8	14.3	90.9	10.9	1.92	10.3	31.2	106.3	8.3	0.49	45.5	0.04	23.0	54.6	4.9	0.18
22	103	F	158.2	3.31	.46	2.09	129.0	12.0	116.6	11.3	2.20	8.5	25.6	107.5	7.8	0.32	43.2	4.55	22.8	67.2	5.0	0.15
23	84	M	168.3	3.33	.42	1.81	127.7	12.6	98.6	11.9	2.07	9.5	26.5	111.3	8.0	0.48	45.0	1.85	24.5	59.0	5.1	1.55
24	69	M	152.5	3.49	.46	1.39	125.3	14.7	95.5	10.1	2.50	11.9	29.4	108.9	8.5	0.51	40.7	0.05	25.4	55.7	4.5	0.06
25	88	F	162.1	5.22	.58	----	134.3	13.2	111.3	12.1	2.40	9.2	35.7	106.2	8.7	0.38	47.7	0.29	24.9	60.1	4.9	0.36
$\bar{X}$	89		157.8	3.94	.53	2.12	129.9	12.8	89.7	11.3	2.65	9.9	29.1	108.6	8.1	0.65	43.3	1.33	23.8	62.2	5.1	1.24
S.E.	2.8		1.43	.13	.05	.08	1.3	0.27	4.06	0.13	0.15	0.21	0.9	1.09	.17	0.07	0.56	0.71	0.8	0.82	.07	0.30

$\bar{X}$ - Sample mean; S.E.- 1 Standard error

RAINBOW TROUT 18 L/6 D, 10°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	113	M	163.9	4.34	.37	2.42	121.5	11.1	124.0	12.6	1.84	8.5	28.4	100.8	7.9	.41	41.7	0.10	27.1	64.0	6.1	0.30
02	130	M	156.8	4.16	.50	2.04	121.2	11.6	-----	12.0	1.64	8.6	35.2	93.6	7.3	.36	36.8	0.08	28.6	67.5	6.2	0.23
03	125	F	167.4	4.49	.50	2.52	123.9	11.1	123.8	12.6	1.78	8.5	32.5	101.5	7.7	.54	40.6	1.70	29.9	6.43	6.2	0.40
04	82	F	151.1	4.83	.54	2.52	123.4	13.9	63.2	12.5	1.59	8.7	37.9	104.3	7.2	.44	44.6	0.84	23.1	66.9	5.8	0.53
05	109	M	153.8	4.21	.66	2.44	118.9	11.6	139.1	12.6	2.00	9.3	32.5	93.6	7.2	.60	41.4	0.42	26.6	68.3	6.3	3.10
06	116	F	156.4	4.29	.62	2.55	128.6	10.9	144.8	12.3	1.83	10.8	28.8	107.4	8.0	.70	50.1	0.20	29.1	64.7	5.9	3.30
07	123	M	158.2	4.16	.50	1.84	127.5	13.3	113.4	12.6	2.21	9.3	34.0	100.7	8.3	.42	38.6	2.97	23.2	70.5	6.3	2.40
08	111	M	156.8	4.31	.54	2.37	123.0	11.6	143.3	12.5	2.20	10.3	31.8	107.1	8.2	.58	44.6	1.78	33.6	66.2	6.6	2.90
09	102	M	162.6	4.34	.71	2.62	127.9	12.0	122.4	11.7	1.98	11.3	34.2	106.5	7.9	.53	40.1	0.60	25.2	67.3	6.1	3.10
10	117	F	155.5	4.06	.71	2.72	116.8	12.2	131.5	12.3	1.45	9.8	27.0	93.5	6.0	.34	37.8	----	22.3	56.1	5.2	0.59
11	133	M	150.7	4.00	.62	2.32	128.4	11.9	128.3	12.5	1.77	10.8	----	95.1	6.9	.63	39.4	2.72	26.1	69.0	6.2	0.88
12	116	F	157.3	4.03	.58	2.02	125.1	10.3	112.2	13.0	1.57	10.2	32.5	96.5	7.9	.40	39.9	0.12	23.7	66.6	6.3	0.82
13	129	M	146.7	4.86	.54	2.09	128.1	11.1	114.6	----	1.54	11.6	31.2	107.5	7.6	.46	44.4	2.35	24.8	66.5	6.1	0.66
14	109	M	151.6	4.73	.58	2.29	123.1	10.4	144.0	11.9	1.28	9.9	33.4	105.2	7.9	.38	46.5	0.87	28.2	74.0	6.6	0.63
15	121	M	156.0	3.83	.62	2.49	117.7	11.0	148.0	12.1	1.61	9.1	35.3	99.4	7.0	.69	48.5	0.92	25.0	61.8	6.0	0.73
16	116	M	154.7	4.68	1.0	2.37	128.6	10.6	85.2	11.6	1.12	9.7	32.3	98.7	7.5	.34	40.1	2.46	31.2	61.2	5.8	0.68
17	104	M	154.0	4.78	.66	2.34	131.9	10.9	134.3	12.4	1.68	9.8	37.6	-----	---	.46	43.5	2.95	30.9	70.1	5.9	0.87
18	81	F	150.7	4.75	.62	2.65	127.3	12.9	110.5	12.2	2.34	7.5	35.7	107.1	7.5	.70	47.4	0.62	28.4	67.8	6.4	2.20
19	120	M	157.3	3.75	.74	2.44	132.3	10.1	142.2	12.9	1.64	9.8	31.8	117.1	8.9	.50	44.0	0.03	27.0	69.4	6.5	0.78
20	92	M	154.2	4.06	.46	2.62	122.5	12.2	101.5	11.6	2.49	7.4	32.8	88.2	5.9	.52	36.3	0.48	31.0	75.1	7.1	0.66
21	141	M	-----	2.76	.29	2.44	127.9	11.3	140.1	12.2	2.16	9.3	35.4	100.1	7.1	.33	46.1	1.45	----	63.6	6.0	0.78
22	82	F	165.2	5.73	.58	2.49	135.9	10.8	134.5	12.3	2.19	10.2	38.7	101.1	6.1	.56	51.1	0.53	35.8	71.9	6.8	1.00
23	58	M	153.8	4.13	.50	2.43	124.2	10.8	78.6	12.0	4.31	9.2	34.0	94.9	4.0	.73	41.9	0.11	29.2	65.4	6.1	0.86
24	81	F	159.0	3.93	.42	2.02	123.9	11.8	131.5	12.3	2.71	9.3	29.7	106.4	8.0	.49	43.4	0.14	28.3	70.7	6.3	0.79
25	119	M	159.5	3.90	.87	2.80	125.1	11.9	145.8	12.2	2.47	9.2	30.9	92.1	6.7	.65	38.6	----	29.2	62.8	5.7	3.40
$\bar{X}$	109		156.4	4.28	.59	2.40	125.4	11.5	123.4	12.3	1.98	9.5	33.1	100.8	7.3	.51	42.7	1.02	27.8	66.9	6.2	1.30
S.E.	4.0		2.7	.11	.03	.05	.91	.19	4.65	.07	.12	.20	.79	1.68	.23	.03	.81	.21	1.11	.84	.08	.21

$\bar{X}$ - Sample mean; S.E.- 1 Standard error

RAINBOW TROUT 18 L/6 D, 18°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	104	F	-----	4.7	.75	2.1	122.2	10.2	-----	12.3	2.9	9.1	36.5	91.1	6.5	0.71	----	1.77	28.8	65.2	6.5	3.60
02	121	M	149.8	5.6	.91	2.8	130.1	10.7	140.4	12.5	2.8	9.1	34.6	107.2	8.0	0.63	45.1	0.05	33.6	66.8	6.8	4.40
03	160	M	148.5	5.0	.71	2.7	118.2	12.4	97.9	12.2	2.7	9.7	32.3	99.2	7.6	0.54	38.6	0.03	25.4	66.1	6.5	2.00
04	103	M	147.6	5.4	.91	2.4	123.4	11.4	119.9	12.6	2.6	9.1	28.9	96.0	7.4	0.77	42.2	0.89	24.6	63.1	6.5	3.80
05	99	F	157.3	4.8	.71	2.6	118.4	12.1	96.2	12.6	1.7	9.8	35.3	101.2	8.3	0.97	45.8	0.06	28.2	57.7	6.2	0.00
06	103	M	152.0	4.9	.79	2.2	124.3	11.1	97.8	12.8	3.9	8.9	38.6	150.4	7.7	0.99	42.3	0.58	28.4	67.0	7.3	0.00
07	109	M	152.0	5.6	.62	2.7	116.7	9.3	144.4	12.6	1.4	8.7	32.9	91.5	6.9	1.20	35.8	0.56	27.1	63.7	6.2	0.22
08	101	M	148.1	5.4	.62	2.4	116.7	9.8	139.0	12.2	1.5	8.3	32.1	99.4	7.9	0.74	40.6	0.58	24.7	61.3	6.3	0.00
09	90	M	143.2	5.9	.62	2.8	124.2	10.6	128.5	12.0	1.5	8.3	31.3	101.2	6.6	0.83	39.5	0.07	34.6	62.3	6.6	0.00
10	223	M	143.2	---	.62	2.4	123.0	10.7	142.8	11.9	1.5	10.0	51.6	82.9	7.0	0.80	38.5	0.02	31.6	65.9	6.5	0.10
11	64	F	141.0	6.3	.58	2.2	124.3	12.2	126.7	11.9	5.3	10.0	36.3	85.3	6.2	0.95	47.0	4.78	24.7	68.4	6.4	0.00
12	87	M	142.8	5.0	.71	2.7	115.6	10.9	145.6	12.0	3.2	10.1	26.7	96.8	7.0	0.71	39.4	0.25	30.4	62.5	6.5	0.00
13	103	F	148.1	5.6	.62	2.5	125.0	10.9	110.0	12.6	2.7	8.9	31.2	96.5	7.6	0.56	38.2	0.05	30.5	64.0	6.9	0.15
14	63	F	147.6	4.9	1.0	2.5	122.2	12.5	129.1	11.4	4.1	9.9	29.9	100.3	6.1	1.13	47.7	0.13	36.8	59.9	6.3	0.00
15	84	M	148.1	6.6	.75	2.3	118.1	10.9	137.2	12.6	3.6	9.6	36.3	89.0	6.9	0.90	48.7	0.30	26.5	65.9	6.1	0.99
16	76	F	147.6	5.8	.37	2.3	114.0	10.7	146.7	11.6	2.8	8.5	33.4	104.0	7.9	0.91	41.8	0.06	41.8	69.0	6.6	2.00
17	87	F	148.1	5.6	.75	2.8	104.6	9.1	145.5	11.6	3.5	7.3	28.7	99.7	7.9	0.65	35.1	1.07	34.5	62.0	6.3	0.96
18	67	F	148.1	6.3	.83	2.9	121.9	10.2	116.5	12.8	3.0	8.6	36.5	92.6	7.3	1.20	44.9	0.02	41.9	69.9	7.1	1.65
19	156	F	158.2	4.6	.91	3.0	121.3	11.0	145.5	11.7	1.3	8.8	38.9	95.9	7.8	0.44	42.6	0.03	27.1	64.1	6.8	0.69
$\bar{X}$	105		148.4	5.4	.73	2.5	120.2	10.9	128.3	12.2	2.7	9.1	34.4	99.0	7.3	0.82	41.9	0.59	30.6	64.5	6.6	1.08
S.E.	8.9		1.4	.20	.03	.06	1.25	.22	4.23	.10	.25	.17	1.2	3.2	.15	.05	.92	.25	1.23	.73	.07	.33

$\bar{X}$, Sample mean ; S.E. ,1 standard error

RAINBOW TROUT 6 L/18 D, 2°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	80	M	155.5	4.1	.50	1.6	136.9	11.6	126.2	11.0	4.0	7.9	28.7	99.1	8.0	0.58	38.5	1.18	25.1	58.1	5.2	3.2
02	125	F	159.0	3.9	.50	1.9	137.9	15.0	129.2	9.4	2.7	8.5	26.7	108.8	8.5	0.39	39.0	7.58	22.3	60.3	5.3	2.3
03	85	M	159.9	3.2	.21	1.8	139.5	12.5	137.4	9.3	2.3	8.7	27.9	120.2	9.7	0.56	41.5	0.63	25.7	64.7	5.2	3.1
04	107	F	160.4	4.6	.58	1.6	125.1	11.2	129.3	10.2	5.1	7.8	24.3	104.1	7.0	0.34	30.5	3.09	23.2	59.4	5.0	2.6
05	76	F	163.0	4.0	.91	1.8	134.9	5.3	122.4	10.9	3.9	7.9	24.8	109.5	9.2	0.74	36.5	6.90	----	65.1	5.8	3.7
06	124	M	162.1	3.5	.58	1.7	133.4	10.6	122.2	10.5	2.5	9.0	26.7	96.8	8.2	0.35	30.5	2.50	22.1	65.1	5.4	1.2
07	103	F	163.4	3.9	---	2.0	129.1	11.5	132.1	10.4	3.7	8.5	21.7	104.4	7.8	0.40	35.9	7.06	25.0	64.7	5.0	1.3
08	99	F	162.6	5.0	.91	---	135.0	11.4	122.9	9.4	4.0	8.0	24.5	99.3	6.8	0.35	40.0	----	24.3	66.3	5.0	1.2
09	102	F	160.8	3.5	.58	1.6	142.9	13.6	94.6	11.9	3.5	---	21.4	98.9	7.6	0.28	31.9	8.24	27.3	68.3	7.6	5.1
10	111	M	149.4	3.7	.17	2.1	145.7	11.6	125.2	11.0	3.1	7.6	24.5	105.1	8.3	0.44	37.5	6.22	21.9	67.3	6.5	4.2
11	96	M	157.3	3.6	.46	1.3	-----	8.6	90.6	----	2.2	7.8	30.5	119.7	10.2	1.30	38.7	5.08	29.1	66.6	7.7	5.0
12	110	M	153.8	3.2	.33	1.4	139.9	16.1	-----	----	4.8	8.2	26.1	104.3	8.0	0.45	42.2	6.30	22.1	64.9	6.8	3.8
13	93	M	156.4	3.0	.54	2.1	137.9	13.4	109.7	11.3	3.3	10.2	26.1	117.8	9.6	0.57	42.9	1.06	28.4	72.4	7.4	5.5
14	87	M	162.2	3.6	1.1	1.7	141.2	12.2	89.8	12.0	1.9	8.5	23.4	109.0	8.1	0.64	37.3	1.21	25.5	70.0	7.5	4.6
15	86	M	160.4	4.3	.42	2.1	131.9	12.6	70.8	11.5	4.9	8.7	25.8	102.3	8.1	0.58	47.3	4.39	29.7	64.8	7.2	4.8
16	82	F	155.1	3.9	1.0	1.8	129.9	11.2	120.2	12.0	3.0	8.7	31.4	115.9	9.5	1.07	38.9	0.15	21.1	61.7	6.3	3.0
17	76	F	157.3	4.6	.29	1.8	128.7	11.1	106.0	10.9	3.7	6.9	24.9	109.7	7.8	1.15	34.6	6.49	20.4	59.2	5.1	1.4
18	90	M	161.7	3.7	.33	1.7	127.4	11.8	112.9	10.9	3.9	8.3	25.1	103.9	7.4	0.39	45.4	5.13	19.1	63.4	5.0	0.8
19	69	M	160.4	4.4	.66	1.3	125.0	11.9	101.2	11.3	2.9	7.8	22.4	92.2	6.4	0.18	30.1	----	21.0	67.8	5.9	3.0
20	59	F	-----	5.3	.71	1.8	-----	9.8	43.9	10.9	---	5.7	25.5	93.6	7.2	1.15	31.5	6.65	17.7	69.5	5.2	1.2
21	60	F	153.8	3.5	.80	1.4	136.0	11.9	105.2	11.7	4.3	8.8	27.7	95.7	8.2	1.10	32.6	3.55	18.8	59.4	4.3	3.6
$\bar{X}$	91		158.8	3.9	.57	1.8	134.7	11.7	109.6	10.9	3.5	8.2	25.7	105.3	8.2	0.61	37.3	4.39	23.5	64.7	5.9	3.1
S.E.	4.0		0.9	.13	.06	.06	1.4	.48	5.15	.19	0.2	0.2	0.6	1.8	.22	.07	1.1	0.61	0.8	.84	.23	.33

$\bar{X}$, Sample mean; S.E., 1 standard error

RAINBOW TROUT 6 L/18 D, 10°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	89	F	153.8	4.8	0.79	2.2	131.3	11.5	112.3	12.4	5.0	7.7	31.1	101.4	7.9	0.45	42.7	3.72	31.9	57.6	5.8	1.80
02	118	F	156.8	4.6	0.50	2.3	125.2	13.1	141.5	12.6	3.0	9.6	28.0	110.8	8.1	0.42	43.3	2.58	22.6	59.3	5.8	2.4
03	123	M	153.8	4.4	0.29	2.0	-----	13.1	138.7	12.1	4.6	8.6	37.2	104.5	7.8	0.49	47.2	3.70	27.0	67.2	5.6	2.10
04	113	F	156.0	4.7	0.75	---	126.4	12.4	128.7	12.9	3.5	7.8	34.8	109.5	8.2	0.39	43.8	4.13	25.1	68.4	6.0	2.00
05	87	F	150.3	5.4	1.20	2.0	113.8	11.7	145.7	12.3	4.0	8.6	37.9	99.1	7.4	0.34	45.2	3.82	29.0	68.0	5.9	1.50
06	203	F	152.5	---	1.20	2.1	118.8	10.8	145.2	10.8	4.8	8.1	25.2	96.5	6.0	0.26	36.2	----	24.5	66.8	6.2	0.88
07	101	F	155.1	4.5	1.30	2.3	122.0	11.5	128.5	12.1	2.1	8.1	33.9	98.5	---	0.55	41.2	7.99	26.9	62.9	5.6	0.84
08	113	F	155.1	4.7	0.87	2.0	120.0	12.7	138.0	11.7	3.0	9.1	29.4	116.0	8.3	0.34	37.3	4.38	20.6	73.5	6.4	0.71
09	110	M	158.6	4.7	0.46	1.6	109.3	9.7	117.9	12.0	2.7	6.5	30.9	106.3	8.5	0.63	----	0.64	20.1	71.9	7.1	3.00
10	94	F	151.6	4.3	0.58	1.9	118.3	11.4	135.8	12.1	2.5	7.8	32.3	108.5	8.9	1.35	36.2	2.01	24.2	71.4	8.1	3.70
11	92	F	152.0	3.9	0.25	1.9	132.4	10.3	113.3	12.1	3.4	9.1	25.9	97.1	6.0	0.36	40.4	2.56	27.0	64.5	7.7	3.90
12	125	M	156.8	5.3	1.00	2.4	126.1	11.3	131.1	11.4	1.9	9.1	30.9	91.7	6.6	0.34	43.6	2.42	24.6	62.5	5.9	1.80
13	100	F	150.7	4.2	1.10	2.5	124.0	10.9	140.4	11.6	3.5	9.2	26.1	104.2	6.6	0.47	40.3	3.47	31.1	62.3	7.3	3.60
14	94	F	141.5	5.1	0.42	1.9	118.1	11.0	127.4	11.7	2.7	8.4	32.4	106.2	7.7	0.54	40.8	9.67	30.1	69.3	8.7	4.20
15	116	M	-----	3.6	0.71	2.4	119.7	12.7	132.7	11.7	2.9	8.6	30.7	99.9	7.3	0.98	----	5.84	20.3	67.4	7.1	2.70
16	94	F	152.9	4.3	0.42	1.7	122.1	10.7	126.1	12.3	2.1	9.5	30.4	99.1	6.6	0.54	42.7	5.80	20.0	59.0	5.7	1.60
17	100	F	149.4	4.4	1.30	2.5	129.0	10.4	127.3	12.1	2.7	8.1	26.4	103.5	7.1	0.27	43.0	7.83	22.4	63.9	6.4	0.57
18	120	M	162.6	3.3	0.54	1.6	124.5	12.1	136.3	12.3	3.6	8.0	28.2	107.6	8.1	0.52	40.7	4.37	29.7	58.7	6.9	3.20
19	104	F	142.4	4.5	0.71	1.9	122.8	12.8	134.7	11.5	2.9	9.0	26.4	99.1	7.5	0.35	38.5	----	22.2	66.7	6.4	0.57
20	103	M	148.1	4.7	0.62	2.4	119.3	12.2	127.7	10.7	3.7	8.3	23.1	101.5	6.3	0.31	38.0	----	21.7	71.7	6.5	2.30
21	121	F	154.7	4.2	0.37	2.5	129.4	10.8	121.9	11.3	4.3	6.9	22.3	92.2	6.4	0.34	----	9.29	24.5	72.7	6.4	2.30
22	105	F	142.4	5.7	0.29	1.8	107.5	10.5	128.1	11.4	2.8	7.8	24.2	105.7	7.0	0.47	----	6.47	26.1	69.4	6.4	2.10
23	96	F	150.0	5.3	1.00	2.3	123.8	11.5	115.6	11.5	3.8	9.0	32.6	96.6	7.2	0.47	42.8	8.89	23.2	77.0	6.7	1.40
24	102	F	160.4	4.9	0.58	1.7	125.6	13.1	104.4	12.4	4.8	8.4	33.9	95.2	7.3	0.89	42.5	4.99	21.6	76.4	7.1	3.00
25	115	F	161.7	4.4	0.96	2.3	130.5	11.9	131.4	11.9	2.0	8.4	27.6	100.9	7.2	0.36	40.0	7.24	26.9	79.6	7.8	1.50
$\bar{X}$	110		152.9	4.6	0.73	2.1	122.5	11.6	129.2	11.9	3.3	8.4	29.7	101.8	7.3	0.50	41.3	5.06	24.7	67.5	6.6	2.10
S.E.	4.5		1.1	.11	.07	.06	1.3	.20	2.1	0.1	.19	.15	0.84	1.2	0.2	.05	0.6	.54	.69	1.2	.17	.21

$\bar{X}$ - Sample mean; S.E.- 1 Standard error

RAINBOW TROUT 6 L/18 D, 18°C

SAMPLE			PLASMA ($mM \cdot l^{-1}$)					SKELETAL MUSCLE ($mM \cdot kg^{-1}$)					LIVER TISSUE ($mM \cdot kg^{-1}$)						CARDIAC MUSCLE ($mM \cdot kg^{-1}$)			
No.	Wt.	Sex	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Na^+	K^+	Mg^{++}	Ca^{++}	Cl^-	Gly.	Na^+	K^+	Mg^{++}	Ca^{++}
01	77	F	155.5	---	1.00	1.7	141.1	12.8	111.5	12.1	5.0	8.7	37.5	103.3	6.5	0.95	50.5	3.43	32.6	62.8	6.2	1.50
02	129	F	155.5	6.0	0.83	2.4	132.4	13.2	135.0	11.7	4.5	8.9	32.9	102.7	7.3	0.39	42.7	4.47	27.1	65.4	6.6	0.97
03	101	F	152.9	4.3	0.83	2.5	119.0	11.8	130.1	11.9	3.3	8.1	32.8	99.2	6.9	0.42	42.9	6.52	24.5	64.5	6.2	1.40
04	107	F	167.0	5.1	0.66	2.2	138.7	12.7	121.4	12.0	3.7	9.9	40.4	96.2	6.5	0.46	42.6	1.31	23.0	51.1	6.5	1.00
05	117	M	153.0	6.3	1.40	2.7	127.1	11.1	136.4	11.3	3.1	8.3	37.7	94.6	5.8	0.68	48.7	6.55	26.6	67.3	6.5	0.99
06	87	M	153.8	6.3	0.66	1.7	124.9	12.5	118.0	12.1	4.7	9.7	35.1	97.0	6.8	0.58	42.0	2.42	----	72.8	7.1	1.20
07	122	M	163.9	4.9	0.58	2.6	139.6	11.7	132.2	11.1	2.6	9.0	37.4	93.2	6.3	0.56	45.0	5.61	22.9	59.9	6.1	0.99
08	108	F	162.2	5.4	0.66	2.0	123.8	12.0	131.3	11.1	2.4	9.8	38.0	93.5	5.1	1.00	41.5	5.05	28.5	68.2	7.2	1.40
09	121	F	146.7	6.2	0.96	---	115.2	12.7	120.4	11.0	1.9	9.1	37.7	97.0	6.8	0.51	45.4	1.53	27.8	71.3	6.8	0.94
10	112	M	159.9	6.3	0.71	2.3	133.8	----	138.3	----	3.0	11.2	41.5	92.3	8.3	1.30	44.0	0.08	29.7	67.4	7.9	2.80
11	84	F	149.8	4.8	0.75	1.9	128.8	10.3	85.1	10.1	3.0	9.7	34.9	91.6	7.2	1.10	42.6	4.51	25.5	67.6	8.4	5.20
12	90	M	150.7	6.7	1.00	2.1	123.6	12.6	107.9	12.0	3.7	9.1	43.1	89.5	5.7	1.30	41.5	7.49	26.4	72.6	7.8	3.10
13	102	F	152.9	6.3	0.71	2.4	124.4	12.1	120.4	11.4	3.6	9.8	35.5	85.2	6.7	1.70	38.3	4.72	26.2	66.6	8.7	3.40
14	102	M	152.9	5.2	1.00	2.5	132.9	11.1	132.4	11.9	3.4	9.0	36.1	96.6	7.6	1.30	43.1	0.89	29.3	62.2	7.4	3.00
15	113	M	153.3	4.6	1.30	2.6	128.0	12.6	129.6	11.6	3.6	9.0	32.8	96.9	6.7	0.54	40.5	6.65	27.2	67.1	7.8	3.00
16	88	M	140.2	5.4	0.42	2.4	-----	11.0	99.5	10.8	3.1	8.5	28.7	94.5	6.8	1.60	----	0.03	22.0	69.6	9.3	4.80
17	113	M	147.2	5.0	1.10	2.1	115.3	10.9	105.0	12.8	---	8.9	33.6	97.4	6.7	0.78	47.8	4.06	27.2	67.6	8.2	3.80
18	124	F	-----	4.8	0.58	2.4	126.3	12.6	118.3	11.6	2.6	9.1	35.8	99.7	6.9	0.78	51.4	0.24	29.8	63.2	8.6	4.50
19	96	F	142.4	4.1	0.71	2.6	109.9	12.5	128.6	11.3	3.0	8.5	30.5	97.6	8.0	1.40	----	0.05	23.6	68.3	7.1	1.00
20	111	M	148.9	4.3	0.60	3.0	126.6	10.9	118.0	11.8	2.5	7.5	41.4	98.9	6.9	0.76	50.0	3.29	29.5	61.2	6.4	0.80
21	108	F	168.7	3.9	0.33	2.8	131.7	13.8	119.8	12.0	2.2	7.8	40.0	100.4	6.1	0.83	50.1	1.00	27.2	69.0	6.5	1.10
22	103	M	155.5	4.8	0.50	2.6	136.2	12.2	115.5	11.9	3.0	8.5	36.4	91.6	6.0	0.61	40.9	5.32	25.0	72.0	9.1	3.90
23	99	F	146.7	5.2	0.33	2.2	110.8	14.3	105.6	12.7	3.8	10.3	39.0	106.1	8.2	1.20	44.5	0.09	30.3	67.4	6.6	1.00
24	92	F	152.5	5.7	0.50	2.6	129.9	15.1	81.9	12.6	4.5	9.5	39.2	109.9	6.7	0.94	50.3	1.25	27.2	69.2	7.0	1.20
25	81	F	143.7	4.4	0.71	2.1	128.5	11.7	92.9	12.2	5.2	10.2	32.2	111.5	6.8	1.40	49.4	0.07	23.4	60.3	6.8	0.88
$\bar{X}$	103		153.2	5.3	0.76	2.4	127.0	12.3	113.0	11.8	3.4	9.1	36.4	97.5	6.8	0.94	45.0	3.06	26.8	66.2	7.3	2.20
S.E.	2.8		1.5	.17	0.05	.07	1.7	.23	5.3	.11	.18	.17	.72	1.2	.15	.08	0.8	.50	0.6	.46	.19	.29

$\bar{X}$ - Sample mean; S.E.- 1 Standard error

RAINBOW TROUT

18 L / 6 D

	2°C %H_2O			10°C %H_2O			18°C %H_2O		
	M	L	%PCV	M	L	%PCV	M	L	%PCV
01	788	744	33.2	756	728	32.0	774	755	40.2
02	758	731	28.5	764	745	42.5	759	769	46.0
03	766	724	35.8	761	744	31.8	757	747	47.5
04	777	732	35.8	771	731	28.5	770	759	44.3
05	766	733	39.0	766	725	33.8	771	758	40.0
06	772	732	35.8	782	734	32.6	775	757	38.7
07	787	717	35.3	773	739	36.8	767	754	50.8
08	770	727	29.0	776	739	33.8	775	757	51.8
09	772	678	37.5	776	745	38.8	773	758	40.5
10	778	711	32.2	771	735	36.0	764	763	39.0
11	779	717	38.4	780	751	33.5	789	753	38.2
12	781	735	36.5	765	733	29.8	774	741	47.4
13	774	719	32.3	778	740	33.2	772	759	46.5
14	761	728	40.0	767	739	37.2	798	758	31.5
15	777	732	25.6	778	749	42.0	774	758	46.0
16	767	756	36.0	767	740	38.3	779	758	45.2
17	782	690	27.5	768	736	42.0	786	763	55.8
18	761	718	30.2	757	738	43.0	772	758	39.0
19	771	716	29.5	772	772	35.5	773	760	48.7
20	752	693	34.0	769	769	40.5			
21	767	719	36.8	764	764	34.2			
22	---	708	28.0	783	783	37.8			
23	763	715	34.6	783	783	31.7			
24	779	679	36.8	774	774	31.5			
25	763	768	42.3	768	768	37.6	---	---	---
$\bar{X}$	771	721	34.0	771	748	35.8	774	757	44.1
S.E.	1.9	4.2	0.86	1.5	3.5	0.80	2.2	1.3	1.30

6 L / 18 D

	2°C %H_2O			10°C %H_2O			18°C %H_2O		
	M	L	%PCV	M	L	%PCV	M	L	%PCV
01	780	777	28.3	770	717	34.7	764	749	44.3
02	779	742	31.3	786	732	33.5	766	736	42.9
03	796	764	24.5	783	743	34.8	766	734	42.2
04	778	706	34.5	783	748	38.7	765	748	49.7
05	779	735	30.0	774	740	33.0	774	734	48.4
06	769	764	32.0	783	720	32.0	774	750	42.5
07	782	729	30.0	775	738	43.8	772	739	42.5
08	785	717	30.4	778	746	32.0	763	739	47.4
09	770	716	33.5	782	752	36.0	776	741	38.3
10	784	735	36.0	785	753	30.5	768	759	37.6
11	786	739	32.5	770	719	30.3	771	739	43.0
12	781	750	34.7	776	724	33.0	768	729	43.5
13	785	767	27.0	768	731	34.0	762	736	43.5
14	783	740	35.0	788	727	35.2	766	748	42.4
15	789	761	31.8	780	731	33.8	768	722	44.3
16	786	773	24.7	779	762	30.4	785	763	41.7
17	799	734	36.0	783	732	39.8	776	763	37.5
18	784	739	32.7	782	747	39.4	780	787	41.5
19	790	715	35.4	783	727	32.5	785	779	36.0
20	791	723	37.6	791	727	35.2	777	754	39.0
21	792	749	35.6	781	729	30.0	779	754	41.0
22				789	731	33.4	775	755	46.7
23				780	736	36.0	782	770	45.0
24				771	746	36.0	777	771	36.1
25	---	---	---	785	738	37.5	805	773	25.2
$\bar{X}$	784	742	32.1	781	736	34.6	774	751	41.7
S.E.	1.6	4.5	0.81	1.2	2.3	0.67	1.9	3.3	0.99

$\bar{X}$ - Sample mean; S.E.- 1 Standard error M - Skeletal muscle ; L - Liver tissue ; %PCV - Hematocrit